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RELATION BETWEEN THE CHEMICAL COMPOSITION OF CITRUS SCALE INSECTS AND THEIR RESISTANCE TO HYDROCYANIC ACID FUMIGATION[1]

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INTRODUCTION

The resistance of certain scale insects on Citrus to hydrocyanic acid fumigation has long been regarded as a problem of great importance, yet relatively little work appears to have been done on it. In the literature there is an almost complete absence of quantitative data on the chemical constituents of scale insects. It is possible that the resistance to hydrocyanic acid fumigation shown by certain scale insects may be related to chemical reactions between hydrocyanic acid and the constituents of the insects. The composition of scale insects is governed to a certain extent by the composition of the parts of the host, which in turn is regulated by the interaction of all the environmental factors. A comparison, therefore, of the composition of the scale with that of the infested portion of the tree will become possible when more is known of the chemical constituents of the scale.

The present paper deals with certain chemical features of citrus scale insects and throws some light on the resistance of the scale to hydrocyanic acid fumigation and on the loss of certain plant constituents by scale infestation.

PREVIOUS WORK

As early as 1922 Quayle (*11*)[2] called attention to the resistance of scale insects in certain localities to hydrocyanic acid fumigation. Not all fumigated sections develop resistant strains, and the areas in which such strains occur are more or less spotted. Instances of resistance or increased tolerance of insects to insecticidal treatment have been reviewed by Boyce (*2*), who found a slight increase in resistance with aphids upon successive fumigations.

Quayle (*11*) found resistance to fumigation to persist even after scale-infested fruits were removed from the tree and fumigated in a different locality. This would indicate that these scale possessed certain differences either chemical or physical, or both.

Gray and Kirkpatrick (*5*) have shown that black[3] and red scale possess increased resistance when subject to "protective stupefaction."

According to Quayle (*11*), the nutrition of the tree appears in some way to be related to the resistance of the scale to hydrocyanic acid fumigation. Red scale were more difficult to kill by fumigation on vigorous thrifty leaves and on vigorous shoots, such as suckers, than

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[2] Reference is made by number (italic) to Literature Cited, p. 491.

[3] A key for the identification of the citrus insects referred to in this paper may be found in a recent bulletin by Quayle (*12*).

on less thrifty leaves and twigs. They were also more difficult to kill on fruit than on twigs or leaves.

Very little is known regarding the mode of action of cyanides on scale insects. Henderson and Haggard (*8*) state that cyanogen compounds are true protoplasmic poisons that exert an inhibiting action upon tissue oxidation, presumably by combining with the catalysts in the living cells of the body tissues containing iron or sulphur. Cyanide poisoning is a form of asphyxia caused by the arrest of internal respiration.

Shill (*17*) found that fumigation of citrus with the dosage of hydrocyanic acid ordinarily used in the field produces an initial increase in respiration (averaging about 75 percent) followed by a return to an approximately normal value after about 35 hours. The respiration, however, in these experiments was carried on under conditions that prohibited photosynthesis.

As suggestions in connection with possible reasons for the tolerance of scale insects to cyanide fumigation, the following antidotes or counteractants should be mentioned. Glucose has been recommended by Violle (*21*) as a protective agent against hydrocyanic acid. Considerable study has been given the freezing-point depression of the sap of citrus leaves by Haas and Halma (*6*), who have shown a quantitative relation of the soluble carbohydrates to the air and soil temperatures throughout the year. Clayton (*3*) injected glucose into tomato plants and found that it counteracted to a large extent otherwise injurious dosages of hydrocyanic acid.

The relation of iron to the resistance of scale insects to fumigation deserves study because of the results obtained by Keeser (*9*) with animals. In an experiment with three groups of rabbits, the first group was fed on rice, the second on greens, and the third on greens together with ferrous chloride. The haemoglobin content of the blood and the iron content of the tissues of the animals in the third group were found to be greater than those of the first group. The third group showed the least susceptibility to the toxic effects of sodium cyanide administered orally. The animals of the second group were more resistant than those of the first group. However, in experiments on the antagonism of iron and cyanide on the respiration of *Paramecium*, Shoup and Boykin (*18*) found that both ferric oxide and cyanide acted in the same manner as when used separately.

Although no data are available regarding the iron content of scale insects, it is thought possible that the hitherto unknown cause of their color differences may in some way be brought about by various compounds of iron.

The difficulty of obtaining accuracy in the analysis of certain insects was pointed out by E. B. Uvarov (*20*). When insects are small, analyses are necessarily carried out on whole specimens, including all the digestive tract, which contains substances not utilized by the insect. The only partial analyses of any importance have been those of insect blood. For a comprehensive summary of the literature on insect nutrition and metabolism, the paper of B. P. Uvarov (*19*) should be consulted.

It may be seen from the investigations mentioned above that in many instances it has not been possible to relate the observations to some definite chemical reaction, except in the case of respiration. Moreover, very few of the workers referred to have utilized scale

insects as their source of material, but have used higher forms of animal life.

In the present investigation the experiments were largely concerned with the inorganic and organic composition of scale insects as related to their ability to resist fumigation. The constituents studied involve, on the one hand, the form and amount of iron and the content of water, ash, phosphorus, sodium, potassium, and copper, and, on the other hand, the content of total nitrogen and sulphur, ieducing sugars, wax, and crude chitin. From the number of scale rnsects present and their composition, some conception is afforded of the loss of plant constituents from scale-insect infestation.

SOURCE OF MATERIAL

The collections of scale-infested fruit or twigs used in the present study were made in resistant and nonresistant areas.[4] The writer has found that 1,000 red scale insects dried at 70° C. weigh 0.0845 g, so that 1 g contains approximately 11,830 insects. The removal of scale from the fruit or twigs in quantities sufficient for analysis, therefore entailed a vast amount of tedious labor. Some samples required an hour or less for their removal from the fruit; others required several hours. In each case the fruit or twigs were wiped free of dust or of dead scale, and the scale insects were removed by gentle scraping with a clean celluloid ruler. They were immediately placed in folded paper envelops and dried at about 60° C.

CHEMICAL METHODS

Sodium was obtained by difference in the chloroplatinate determination of potassium. Total phosphorus was determined by the use of magnesium nitrate, precipitation as phosphomolybdate, and later as pyrophosphate. After the removal of the phosphate, total iron was determined by the thiocyanate method as used by Elvehjem and Hart (*4*). Inorganic iron was dissolved from the dry powder by the use of 20-percent sulphuric acid, the excess acid being removed in a Kjeldahl flask and the iron fractions determined separately or by difference from the total iron. Total and reducing sugars were determined by the Shaffer and Hartmann (*15*, *16*) method after the solution was cleared. In the absence of a sufficient sample their micromethod may be used.

Manganese was determined by the periodate method, as used by Samuel and Piper (*13*), while copper (*1*) was determined by the iodide method (*14*), 0.1 N thiosulphate freshly diluted to 0.0020 N being used. The wax content was determined by placing a weighed sample of the scale in a weighing bottle and extracting the wax by means of anhydrous ether. This process was continued for 10 days, with recourse to occasional shaking. The scale insects were then collected in porous-bottom crucibles, thoroughly washed with ether, dried at 70° C., and weighed, the wax being determined by difference.

Crude chitin (*7*) was prepared by first extracting the wax with ether, then boiling the scale with half-saturated caustic alkali until they were white, after which they were separated from the solution by means of porous-bottom crucibles. They were then washed with

[4] These collections were made by the author or through the kindness of agricultural inspectors of the agricultural commissioners' offices or of other interested friends.

water, which was followed by dilute acid to neutralize the alkali and remove iron salts. Finally, the crude chitin was washed with alcohol and ether, dried at 70° C., and weighed.

ANALYTICAL RESULTS

The ash, iron, inorganic (ash) phosphorus, and manganese content of the red scale *Aonidiella aurantii* (Mask.) on lemons is given in table 1. At the suggestion of H. S. Fawcett, of the California Station, the results for the samples collected in 1931 and 1932 were compiled in one group which was subdivided into resistant and nonresistant insects. Similarly, the samples collected in 1933 were compiled in a second group, which also was subdivided into resistant and nonresistant insects. The samples of scale insects represent a good cross section of both the resistant and the nonresistant areas for red-scale insects on lemon. The collection of samples in 1931–32 required several months; in 1933 only a few weeks were required.

TABLE 1.—*Ash, iron, manganese, and inorganic (ash) phosphorus content of the dry matter of mature red scale insects on lemons as related to fumigation resistance*

Fumigation resistance of scale	Place of collection	Date	Weight of dry-matter sample used	Ash in dry matter	Quantity in dry matter		
					Fe	Inorganic (ash) P	Mn
			Grams	*Percent*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Resistant	Arlington	June 26, 1931	1.1671	13.25	4,798	.....	68.5
	Do	May 1931	3.4170	.....	.....	.....	190
	Orange County	July 3, 1931	.7944	10.08	7,427	4,179	30
	West Riverside	July 26, 1931	.4107	.....	7,061	5,095	
	Yorba Linda	Jan. 12, 1932	.5887	11.35	1,868	3,501	
	East Whittier	Jan. 7, 1932	.1973	16.73	2,471	3,529	
	Yorba Linda	Jan. 12, 1932	.7755	10.97	3,750	3,268	
	East Whittier	Jan. 7, 1932	.1956	18.35	1,534	3,987	
	La Habra Heights	Jan. 19, 1932	.4974	8.40	3,229	3,751	
	Yorba Linda	do	.4864	7.98	3,739	3,722	
	La Habra	do	.3205	9.95	1,092	3,041	
	Arlington	do	.4150	5.40	934	5,704	
	La Habra	do	.2800	13.90	3,125	3,481	
	Yorba Linda	do	.4169	9.19	959	4,075	
Average				11.29	3,230	3,944	
Nonresistant	West Riverside	Aug. 2, 1931	0.4848	.....	4,125	5,225	
	Covina	October 1931	2.1032	5.28	2,140	7,054	24
	Do	do	1.2447	4.99	3,414	6,368	20
	Anaheim	Jan. 19, 1932	.1598	17.84	5,944	3,834	
	Olive	do	.3850	16.32	3,896	3,545	
	El Cajon, San Diego	Jan. 7, 1932	.3052	5.44	532	4,289	
	San Dimas	Jan. 6, 1932	.4549	8.64	2,699	5,020	
	Covina	October 1931	.4875	6.01	2,290	6,113	
	Anaheim	Jan. 19, 1932	.2407	11.09	4,600	4,165	
	El Cajon, San Diego	Jan. 16, 1932	.3551	5.47	668	4,784	
Average				9.01	3,031	5,040	
Resistant	Corona	Aug. 22, 1933	0.1952	.....	1,946	17,552	
	Do	do	.3430	.....	1,865	9,262	
	Do	do	.1367	.....	2,167	24,480	
Average				.....	1,993	17,098	
Nonresistant	Escondido	Aug. 28, 1933	0.1557	.....	12,455	13,240	
	Montecito	do	.1632	.....	1,912	19,128	
	Chula Vista	July 27, 1933	.1115	.....	13,993	20,000	
	La Mesa	do	.1006	.....	2,465	22,440	
	Chula Vista	Aug. 22, 1933	.1106	.....	6,328	12,018	
	Do	do	.1331	.....	4,957	19,884	
	Do	Aug. 24, 1933	.1585	.....	4,038	15,828	
Average				.....	6,593	17,505	

The average ash content of the dry matter was greater in the resistant scale insects, although the variations in both cases were large. The average iron content for the 1931–32 period was 3,230 parts per million for the resistant and 3,031 parts per million for the nonresistant scale insects, while for the 1933 period the average content was 1,993 parts per million for the resistant and 6,593 parts per million for the nonresistant scale insects. In the 1931–32 period the average ash phosphorus content was much greater in the nonresistant insects, while in the 1933 period the average for both resistant and nonresistant scale insects showed little or no difference. From these data it may be concluded that total iron bears no relation to the resistance of the insects to fumigation. The number of manganese determinations was limited, but among these the higher values were associated with the resistant insects.

In view of the almost complete absence of such analytical data in the literature, the results in table 1 have value in giving some conception of the iron, phosphorus, and manganese in red scale insects, and the marked variations that may occur in various years. The cause of such variations, whether a result of climatic or other factors, remains to be determined. The content of these constituents in scale insects was compared with data obtained by Haas and Klotz [5] for the infested portion of the citrus tree. In every case the dry matter of the scale insects contained far more of these ash constituents than did the infested portion of the citrus tree.

Determinations of the ash constituents were made on several samples of yellow scale (*Aonidiella citrina* (Coq.)) obtained from navel oranges, on four samples of red scale, two of which were obtained from Valencia oranges, one from St. Michael oranges, and the other from grapefruit, and on a single sample of purple scale (*Lepidosaphes beckii* (Newm.)) from navel oranges. The iron and phosphorus content of yellow and purple scale insects differs but little from that of red scale insects. The manganese content of the nonresistant yellow scale insects is the same as that of the nonresistant red scale insects in table 1. The ash content of the resistant red is greater than that of the nonresistant red scale insects, a finding which agrees with the data in table 1. The data for the average iron and phosphorus content of red scale insects collected in 1932 from oranges (table 2) agree with those for red scale insects collected in 1931–32 from lemons (table 1), in that the nonresistant red scale insects contain relatively much more phosphorus in relation to iron than the resistant red scale insects.

Table 3 shows the data obtained for Latania scale (*Aspidiotus lataniæ* (Sign.)) collected from the bark of twigs or branches of avocado, and for red, black (*Saissetia oleae* (Bern.)), and cottony-cushion (*Icerya purchasi* (Mask.)) scale from various portions of citrus trees. The ash was least in the cottony-cushion and greatest in the red scale insects. The black scale contained considerably less iron, but more ash phosphorus than the red scale.

[5] HAAS, A. R. C., and KLOTZ, L. J. PHYSIOLOGICAL GRADIENTS IN CITRUS FRUITS. 1934. (Unpublished manuscript.)

TABLE 2.—*Ash, iron, manganese, and inorganic (ash) phosphorus content of the dry matter of mature scale insects on oranges and grapefruit as related to fumigation resistance*

Fumigation resistance of scale	Type of scale	Mature fruit infested	Place of collection	Date	Weight of dry matter sample used	Ash in dry matter	Quantity in dry matter		
							Fe	Inorganic (ash) P	Mn
					Grams	*Percent*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Nonresistant	Yellow	Navel orange	Redlands; multiplied at Citrus Experiment Station insectary.	Apr. 26, 1932	0.2306		3,523	5,249	
			do	do	.2500		2,950	4,652	
			do	do	.2115		4,965	3,949	
			Highland	Nov. 1, 1931	2.0588		2,793	4,998	24
		Average					3,558	4,712	
Resistant	Red	Valencia orange	Yorba Linda	Jan. 19, 1932	.4383	18.25	3,879	3,113	
				Jan. 12, 1932	.5376	12.20	4,887	3,108	
		Average				15.23	43,383	3,111	
Nonresistant	Red	St. Michael orange	Citrus Experiment Station, Riverside.	Jan. 19, 1932	.4317	4.61	1,730	5,032	
	do	Grapefruit	West Riverside	Aug. 2, 1931	2.2642		3,445	5,389	
	Purple	Navel orange	Olive	Jan. 19, 1932	.6760	12.90	5,594	2,101	

TABLE 3.—*Ash, iron, manganese and inorganic (ash) phosphorus content of the dry matter of various scale insects on bark as related to fumigation resistance*

Fumigation resistance of scale	Type of scale	Location of scale infestation on host	Place of collection	Date	Weight of dry matter sample used	Ash in dry matter	Quantity in dry matter		
							Fe	Inorganic (ash) P	Mn
					Grams	*Percent*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Nonresistant	Latania	Large avocado branches	La Habra	Jan. 19, 1932	0.6554	10.82	1,602	2,805	
		Small avocado twigs	Irvine	do	.3844	8.22	586	4,347	
		Average				9.52	1,094	3,576	
Resistant	Red	Lemon branches	Yorba Linda	Jan. 19, 1932	.4898	12.19	4,378	3,525	
Nonresistant	Red	Lemon branches	Near Orange, South of Anaheim	do	.6813	22.78	6,592	3,638	
Do	Black	Valencia orange twigs	Anaheim	May 9, 1932	.2902		793	5,720	
					.2610		748	5,030	
					[1].3927		510	3,925	
Do	Black	Peppertree twigs	Riverside	Nov. 17, 1931	[2]23.9314		927	4,344	20
					11.6922				
		Average					745	4,755	
Do	Cottony-cushion	King orange twigs	Citrus Experiment Station variety orchard.	Oct. 29, 1931	[2]3.7325	2.58	648	5,585	10
		Grapefruit trunk (1 foot above ground).			1.8920				
				do	[2]1.4640	5.12	2,105	3,886	
					.8931				
		Average				3.85	1,377	4,736	

[1] Many young.

[2] Fresh weight.

The fresh sample of black scale insects collected from pepper tree twigs contained 51.14 percent of water, that of cottony-cushion scale insects on King orange twigs contained 49.31 percent and on grapefruit trunk 39.00 percent. The percentage of water in red scale insects obtained from lemons, as shown in table 4, was only 35.71 percent. The average total sulphur content of the dry matter of these red scale insects was 0.20 percent, or about one-third that of the total phosphorus content, while the average total nitrogen content was 6.59 percent, or 65,900 parts per million. Haas and Klotz [6] found the total nitrogen content of Valencia orange juice to be approximately 1,600 parts per million. The large content of nitrogen, iron, and phosphorus in scale insects accounts in part for the permanently discolored sunken areas in and surrounding the position of the scale.

TABLE 4.—*Total N, S, and P content of the dry matter and H_2O content of resistant red scale insects from large green lemons at Corona, Calif., 1933*

Constituents	Date of collection	Weight of dry matter sample used	Constituent of dry matter	Average
		Grams	*Percent*	*Percent*
Total N	Sept. 25	2.0446	6.53	6.59
		1.1174	6.53	
		1.1939	6.65	
		1.5421	6.65	
Total S	Oct. 15	1.2822	.21	.20
		2.4801	.19	
Total P	Oct. 15	1.2822	.634	.652
		2.4801	.670	
H_2O	Oct. 14	.2610	[1] 35.71	

[1] Percentage of fresh weight.

When scale infestation is heavy, the resulting drain on the nutrients of the tree may become so great as to decidedly weaken it or even to kill certain portions. Those who fumigate trees frequently ascribe hydrocyanic acid injury to weakness of the trees. Many factors, such as excessive soil moisture and heavy scale infestation, may bring about such a condition. Attention may be called to a possible temporary decreased fumigation resistance of citrus twigs shortly after they have produced new shoots. During the growth of these shoots there is a depletion of the inorganic constituents of the mother twigs (*6*). No doubt there is also a depletion of the soluble carbohydrates and organic reserves, which may account for the reduced resistance until new supplies are brought in. It is possible, therefore, that a depletion of inorganic and organic matter from shoots makes them less resistant to fumigation, while the scale insects become more resistant, depending upon the nature of their food supply. Thus, if scale insects contain large amounts of reducing sugars, it is conceivable that they may be less easily killed by fumigation, and if they contain large amounts of copper it is conceivable that they may be more easily killed by fumigation. In any event, the resistance of the tree must also be taken into account.

In addition to determinations of the iron and phosphorus content, determinations were also made of the copper content of red and black scale insects. The results reported in table 5, show that considerable

[6] HAAS, A. R. C., and KLOTZ, L. J. See footnote 5.

copper may accumulate in scale insects. It is known that copper occurs in the blood of certain insects. Here, however, it cannot be said whether the copper is located in the scale blood (if such exists), body structural parts, or body fluids, or whether it occurs simply as ingested fruit juices in the digestive tract. In any event analyses now being made of citrus leaves and fruit show definitely the presence of copper, and its absorption by scale insects appears as a logical sequence.

TABLE 5.—*Iron, inorganic (ash) phosphorus, and copper content of the dry matter of red and black scale insects*

Type of scale	Portion of host infested	Place of collection	Weight of dry matter sample used	Quantity in dry matter		
				Fe	Inorganic (ash) P	Cu
			Grams	*P.p.m.*	*P.p.m.*	*P.p.m.*
Red	Mature lemons	La Canada	0.4185	1,840	2,118	585
	Green lemons	Glendora	.3119	5,290	1,446	673
	Silver lemons	Corona	4.8790			65
Black	Navel orange twigs	Victoria Avenue, Riverside	1.1510	2,780	4,400	365

Citrus trees are frequently treated with copper sulphate in order to cure "exanthema." This process, when followed too soon by cyanide fumigation, may not only bring about the premature abscission of many leaves, but may also badly scorch the fruit.[7] This effect of copper in increasing the toxic action of cyanide fumigation suggests that possibly cyanide-resistant scale may contain less copper than less resistant scale. This hypothesis receives some confirmation from the fact that the red scale at Corona, which are regarded as resistant to cyanide fumigation, contained the lowest concentration of copper found in the present experiments.

In view of the fact that a careful search of the literature revealed no data on the reducing-sugar content of scale insects, and in view of the known action of reducing sugars such as glucose as an antidote for cyanide, considerable effort was made to obtain some data on both resistant and nonresistant red scale insects collected from lemons. The results are given in table 6, where it is seen that the average reducing-sugar content of resistant red scale insects is greater than that of nonresistant scale insects. If further studies should confirm these preliminary results, it would seem desirable to make a study of temperature and other relations of certain resistant and nonresistant locations upon the reducing-sugar content of scale insects.

The results for the different forms of iron in resistant and nonresistant red scale insects given in table 6 show that most of the iron is present in the inorganic form. The removal of such large amounts of iron necessarily must deplete the supply of iron ordinarily available for chlorophyll catalysis.

In the sample of red scale (0.4103 g of dry matter) obtained from lemons collected at Covina in October 1931, the water-soluble iron was 302 parts per million, while the insoluble iron was 1,706 parts per million. In another sample of these same scale insects (0.3918 g of

[7] HAAS, A. R. C., and QUAYLE, H. J. COPPER CONTENT OF CITRUS LEAVES AND FRUIT IN RELATION TO EXANTHEMA AND FUMIGATION INJURY. 1934. (Unpublished manuscript.)

dry matter) the iron soluble in 95 percent alcohol amounted to 1,174 parts per million while the insoluble iron was 1,149 parts per million. If the marked variation in the iron content of the various samples is taken into account, there appears to be no relationship between the form of the iron and the resistance to fumigation.

In table 7 are given the results obtained for the forms of iron in black, yellow, and purple scale insects. Again we note that most of the iron of scale insects is in the inorganic form. Further determinations of the reducing-sugar content of scale insects are reported in table 7. The nonresistant yellow and purple scale insects show a lower reducing-sugar content than the resistant black scale insects. The yellow scale insects were obtained from Washington navel and other navel oranges that were overmature, so that they had had ample opportunity to build up a large reducing-sugar content. Despite this fact, the data for reducing-sugar content of nonresistant red, yellow, and purple scale insects (tables 6 and 7) agree in being lower than those for the resistant red and black scale insects.

TABLE 6.—*Organic and inorganic iron, reducing sugar, and total sugar content of the dry matter of red scale insects from lemon fruits and twigs as related to fumigation resistance*

Fumigation resistance of scale	Portion of host infested by scale insects analyzed	Place of collection	Date	Weight of dry matter sample used—		Quantity in dry matter			
				For iron	For sugar	Organic iron	Inorganic iron	Reducing sugars	Total as reducing sugars
				Grams	*Grams*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Resistant	Mature fruit	Arlington	Jan. 19, 1932	0.5043	1.4769	214	1,521	26,170	32,230
		Corona	Aug. 22, 1933		.5058			22,900	
		Yorba Linda	Jan. 19, 1932	.4307		1,080	4,256		
		do	do	.0979		725	4,698		
		La Habra	do	.7757		709	2,407		
		Average				682	3,221	24,535	
Nonresistant	do	Covina	October 1931		2.0291			10,320	14,632
		Chula Vista	July 27, 1933		.2157			14,900	
		do	Aug. 22, 1933		.4674			19,300	
		do	do		.5700			11,500	
		San Dimas	Jan. 6, 1932	.5128		683	2,471		
		El Cajon	Jan. 7, 1932	.2649		495	1,397		
		Anaheim	Jan. 19, 1932	.1405		854	5,694		
		Covina	October 1931	.7948		287	881		
		Average				580	2,611	14,005	
	Twigs	Anaheim	Jan. 19, 1932	.3106	.9408	1,771	5,634	16,178	20,886

TABLE 7.—*Organic and inorganic iron, reducing sugar, and total sugar content of the dry matter of black, yellow, and purple citrus scale insects as related to fumigation resistance*

Fumigation resistance of scale	Type of scale	Portion of host infested	Place of collection	Date	Weight of dry matter sample used		Quantity in dry matter			
					For iron	For sugar	Organic iron	Inorganic iron	Reducing sugars	Total as reducing sugars
					Grams	*Grams*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Resistant	Black	Thompson Navel orange twigs	Charter Oak	June 1, 1932	0.2066	0.5278	281	581	22,925	32,399
				June 10, 1932	.5242	.4867	668	2,036	29,998	48,079
		Average					475	1,309	26,462	40,239
Nonresistant	Yellow	Overmature navel oranges	Redlands; multiplied at Citrus Experiment Station.	Apr. 26, 1932	1.0713	2.2373	978	1,080	14,410	20,140
	Purple	Navel oranges	Olive	Jan. 19, 1932	1.0258	2.0952	1,102	1,884	6,396	9,374

In addition to the iron, phosphorus, manganese, copper, and reducing sugar content of scale insects, determinations were made of the wax, alcohol-soluble matter, and crude chitin content of resistant and nonresistant red scale insects. The results, given in table 8, show that the nonresistant contained more wax than the resistant scale insects on lemons. No work has thus far been carried on to indicate either the manner or the extent to which the wax acts in protecting the scale from fumigation or other toxic agents. Metcalf and Hockenyos (*10*) found that the wax is specific for each species of scale and that the hard covering of armored scale insects is an intimate mixture of wax with some other material. In the case of the oyster-shell scale (*Lepidosaphes ulmi* L.) they found that after the wax had been removed the remainder of the scale contained no chitin.

TABLE 8.—*Wax, alcohol-soluble matter, and crude chitin content of the dry matter of citrus red scale insects from mature lemons as related to fumigation resistance*

Fumigation resistance of scale	Portion of host infested	Place of collection	Date	Weight of dry matter sample used	Quantity in dry matter		
					Wax	Soluble matter in 80 percent alcohol at 80° C.	Crude chitin
				Grams	*Percent*	*Percent*	*Percent*
Resistant	Mature lemons	Corona	Aug. 22, 1933	0.0997	8.83	10.73	24.47
				3.1485	10.51	11.98	21.48
				.0582	11.17	10.82	23.37
Average					10.17	11.18	23.11
Nonresistant	Mature lemons	Chula Vista	July 27, 1933	.1261	13.64	9.83	33.78
		La Mesa	do	.0427	21.78	8.66	18.50
		Chula Vista	Aug. 22, 1933	.0325	16.92	3.69	24.92
		do	do	.0486	16.67	9.26	26.54
		do	do	.0641	15.13	9.36	24.33
Average					16.83	8.16	25.61

In the present analyses, therefore, after the wax was removed, the same samples of scale were kept in 80 percent alcohol at 80° C. overnight, and were then washed with warm 80 percent alcohol, dried, and weighed. Table 8 shows that higher values were obtained with the resistant than with the nonresistant scale insects, a finding which is in harmony with the results reported in table 6, since 80 percent alcohol is a good solvent for reducing and other sugars.

Following the removal of wax and substances soluble in alcohol, determinations of crude chitin were made. Table 8 shows that the percentage of crude chitin is about the same in all the insects. In most cases this substance was contaminated with iron; so it is possible that iron and other salts are integral parts of the wax or chitin.

Figure 1 shows the remains of a red scale insect boiled in caustic alkali until white. Metcalf and Hockenyos (*10*) found the Florida red scale insect to consist of five distinct increments. It is possible that much of the material reported as chitin in table 8 may in reality be some form of wax, for it is known that waxes are not acted upon equally by all solvents, and some investigators consider the mouth parts and the lobes, plates, and spines of the pygidium as the only

really chitinous parts of a red scale insect. Hence the ether used as a wax solvent for 10 days on the scale insects reported in table 8 may have removed only part of the wax. The results, however, for crude chitin in table 8 would indicate that at least an equilibrium had been reached with ether as a wax solvent.

Determinations of sodium and potassium made on large samples of yellow, purple, and red scale insects are reported in table 9. Although more determinations are to be desired, those that were made show a

FIGURE 1.—Remains of a citrus red scale insect after treatment with a boiling solution of strong alkali. The open space in the center was caused by the falling out of the part of the armor developed during the first instar of the insect.

uniform concentration of potassium in red and purple scale insects and nearly double this concentration in yellow scale insects. The purple scale insects contained the largest and the red scale insects the smallest percentage of sodium. If further determinations of potassium confirm these preliminary results they will furnish a chemical method of distinguishing yellow from red scale insects, two species which resemble each other closely.

TABLE 9.—*Sodium and potassium content of the dry matter of yellow, purple, and red scale insects*

Scale	Ripe fruit infested	Place of collection	Date	Weight of dry matter sample used	Quantity in dry matter	
					Na	K
				Grams	*P.p.m.*	*P.p.m.*
Yellow	Overmature navel orange	Redlands; multiplied at Citrus Experiment Station insectary.	Apr. 26, 1932	1.5531	3,171	5,024
Purple	Navel orange (scale-infested calyx half, green; scale-free stylar half, ripe).	Olive	Jan. 19, 1932	.4705	9,711	2,663
Red	Lemon	Arlington Heights	do	2.4431	1,755	2,897
				1.6500	2,217	2,638

SUMMARY

The composition of various citrus scale insects was studied in relation to the composition of the part of the tree that they infested and also in relation to the resistance of the scale to cyanide fumigation. Since no previous work appears to have been done in this field of study, the results must necessarily be considered as preliminary.

Citrus scale insects accumulate a much greater concentration of a given constituent within themselves than occurs in the portion of the affected host tree.

No relation was found between the ability of the insects to resist fumigation and their organic or inorganic iron or (ash) phosphorus content.

Citrus scale insects contain considerable copper. A reduced content of copper may be related to fumigation resistance.

Among other possible relations brought out by the analyses were those involving the amount of wax and the concentration of reducing substances in the scale insects.

A considerable portion of a scale insect consists of material that is soluble in a boiling solution of strong alkali.

The concentration of total nitrogen in red scale is considerably higher than that of total sulphur or total phosphorus. The concentration of potassium in the yellow scale examined was nearly double that in the red scale.

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BACTERIAL BLIGHT OF CARROT [1]

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INTRODUCTION

In May 1931 a disease, apparently bacterial in nature, was observed on the leaves, petioles, stems, and floral parts of carrots (*Daucus carota* L. var. *sativa* DC.) in the extensive seed-producing area of the upper delta region of the Sacramento Valley, Calif. In many cases there was a severe blighting of the flower clusters (umbels) just previous to or shortly after floral development.

The disease again appeared in the late spring of 1932 in practically every carrot-seed field in that district and was also observed on seed carrots growing on the university farm, Davis, Calif., from stecklings (medium-sized carrot roots used for seed production) secured from the region mentioned above. In 1933 the disease was observed early in May in a limited number of plantings, but its further development was apparently arrested by dry weather and no appreciable damage was done to the seed crop.

In California carrot seed is planted in August and September in double rows on raised beds or in flat 15-inch rows, commonly called root beds, where, forced by frequent irrigation, medium-sized roots (Stecklings) are produced by November and December. The stecklings are then dug and transplanted to the seed fields for commercial seed production. Early in December 1932 the disease was found near Sacramento in carrot-root beds just before the stecklings were transplanted to the seed fields and in commercial carrot fields which had been grown from seed. The disease was again found in this same area late in November 1933 in carrot-root beds and commercial plantings in fields where carrots had not been grown in 1932.

SYMPTOMS OF INFECTION ON LEAVES, PETIOLES, STEMS, AND FLORAL UMBELS

The disease is characterized by irregular necrotic spotting of the leaves, dark-brown linear lesions on the petioles and stems, and a blighting of the floral parts of the plant. The first evidence of the disease is the presence of small, irregular, yellow areas on the small leaf segments (fig. 1, *A*), which in a few days, under favorable conditions, become more or less well defined dark-brown, water-soaked spots. The darkened central part of the lesion soon becomes dry and brittle and often is surrounded by an irregular yellow halo (fig. 1, *C* and *D*). Often infection takes place along the margin of the ultimate leaf segments and causes a lateral curling of the leaflet (fig. 1, *B* and *C*).

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[2] The writer wishes to express his appreciation to Profs. Ralph E. Smith and Max W. Gardner for helpful suggestions and criticism, and to O. C. Whipple for valuable assistance in the pathogenicity studies.

In the field the disease on the leaves is frequently confined to the tips of the segments, presumably because moisture collects on the drooping tips and affords favorable conditions for infection. As a result of this type of infection, the tips are killed and a characteristic yellow area is evident immediately back of the dead tissue (fig. 1, *E*).

In some cases there is no definite margin between diseased and healthy tissue, and the characteristic halo around the dead tissue is absent. This usually occurs under conditions extremely favorable

FIGURE 1.—Leaf symptoms of carrot bacterial blight: *A*, Incipient infection showing as irregular chlorotic areas on the leaflet; *B* and *C*, marginal lesions resulting in a lateral curling of the leaflets; *D*, necrotic spots surrounded by halos; *E*, killing of segment tips and the resulting chlorotic area back of each dead tip.

for infection, and once the tissues are invaded, they quickly become dark brown and water-soaked (fig. 2, *A* to *G*). It is not unusual to find entire segments and leaflets invaded and killed (fig. 2, *A* and *C*–*G*, and fig. 3). This type of infection is often more prevalent on the lower leaves in root beds (fig. 4, *A*) and under artificial greenhouse conditions, presumably where transpiration is retarded and the leaves are turgid. Many of the lower leaves in older plant beds are often killed and dry up. The lower leaves of plants in the seed field often

show severe necrosis, resulting in the death of many segments and leaflets and a yellowing of the uninvaded tissue (fig. 5). In severe cases of the disease, all the upper leaves have a yellowish chlorotic appearance and many of the lower leaves are killed.

With severe leaf infection, the infection usually extends into the petiole and stem, causing conspicuous dark-brown, water-soaked, linear lesions on these organs. Often abundant gummy bacterial exudate collects on the leaf petiole and stem and may run down the stem for a distance of several inches.

Under artificial greenhouse conditions, the disease appears on the leaves as irregular yellow areas with minute irregular dark-brown spots

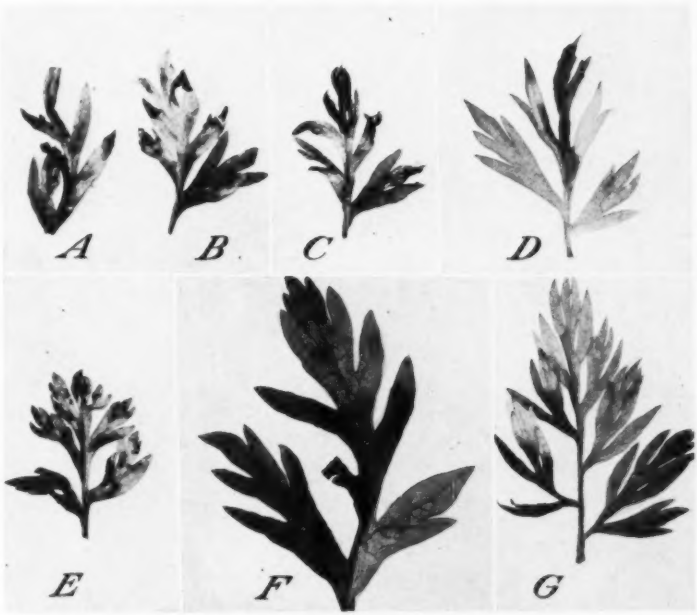

FIGURE 2.—*A-G*, Leaf symptoms of carrot bacterial blight which occur under very favorable environmental conditions, showing segment tips, segments, and leaflets killed. Under such conditions, where invasion of the tissue is rapid, the halo area around the necrosis is often absent.

in the central area of the chlorotic region (fig. 6). Numerous small incipient lesions along the margin of a segment result in the death of the entire margin or the whole segment, and produce a yellowing of the remainder of the leaflet (fig. 3).

In the later stages of the plant's development, the disease is perhaps more conspicuous on the floral parts than on the leaves. Infection usually takes place just before or soon after the umbel expands. If infection occurs either before the developing umbel emerges from the leaf sheath or after it emerges from the sheath, but before it opens up, the entire umbel is usually killed. Where infection is delayed until after the umbellets have opened, many of them may escape the disease. Infected umbellets may be scattered irregularly in the umbel (fig. 7,

B), or the outer umbellets may be blighted while a few healthy ones remain in the center (fig. 4, *D*). More often infection is confined to one side of an umbel, and the disease frequently extends into the peduncle and causes the umbel to be curved downward on the infected

FIGURE 3.—Carrot leaflet segments showing death of an entire segment and necrosis along the margin of the two adjacent segments. Incipient lesion and resulting chlorosis on the inner margin of the segment on the left. ×12.

side. The disease also causes necrotic spots on the involucral bracts which are often so extensive as to kill these bracts (fig. 7, *C*, *D*, *E*).

A copious gummy exudate may collect around the bases of the flowers (raylets) of infected umbellets, and in severe cases of the disease the entire umbel becomes encased in a mass of this exudate.

It is not unusual to find the gummy exudate actually running down the peduncle to the stem.

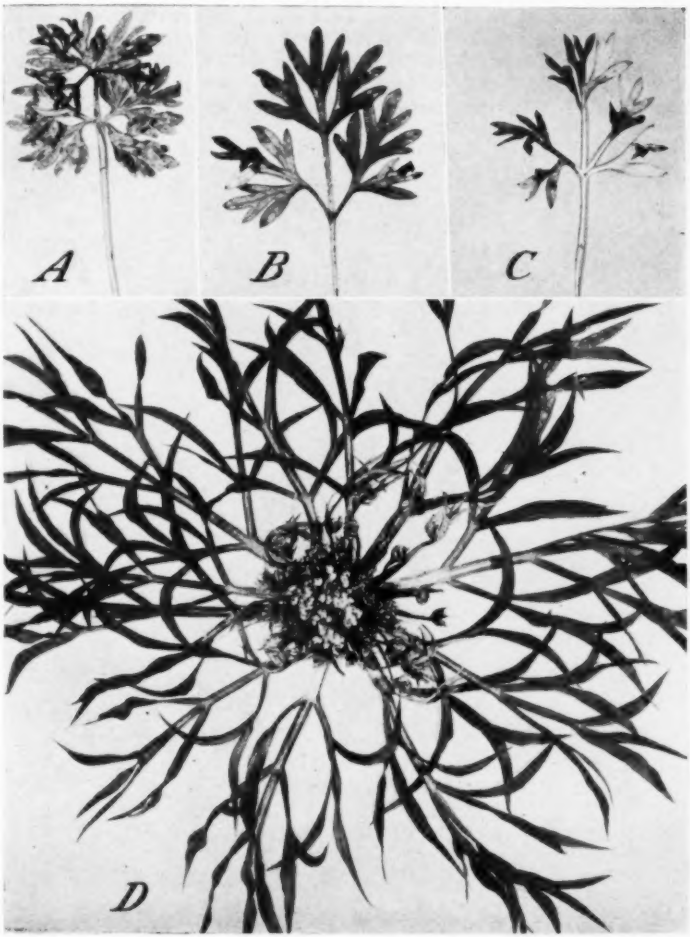

FIGURE 4.—*A*, Infected leaf from a young plant in a commercial root bed; *B* and *C*, leaves from young plants grown in steam-sterilized soil from seed previously inoculated by immersing in a water suspension of the bacterial blight organism and drying thoroughly before planting; *D*, vertical view of diseased umbel showing blighting and death of the outer umbellets and healthy, later appearing, central umbellets.

DAMAGE TO SEED CROP

While the disease as it occurs on the leaves and stems no doubt materially weakens the plants in their development, the principal damage, as it has been observed in California, is caused by a blighting of the seed umbels. However, if the disease should become prevalent in the commercial carrot root-growing districts where the roots are

marketed with the leaves attached, serious losses would be incurred because of the unsightly necrotic spotting of the leaves.

Normally there is a considerable variation in the time at which individual plants in the same field start to develop seed stalks. Many plants begin branching and producing flower umbels early in the spring, while others in the same field do not begin floral development until late spring or early summer. In many plantings the disease kills a large percentage of the early appearing umbels which

FIGURE 5.—Carrot leaf showing typical severe necrotic blighting of segments resulting from natural infection under field conditions.

are on the terminal shoots. If the attack occurs early and in a severe form, the plant is so weakened that it seldom produces any seed. Ordinarily, however, new branches are developed from below and a partial seed crop is produced on the lateral shoots. If the terminal shoot is killed early, the development of the main stalk is arrested and the number of lateral shoots which develop from the main stalk are greatly limited, with a consequent material reduction in seed production. Although the primary damage may often be confined to

the early terminal umbels, yet if favorable weather conditions, such as relatively high humidity and moderately low temperature, continue, later-appearing umbels on the lateral shoots are attacked and partially or entirely destroyed.

During the spring of 1931, considerable damage from this disease was sustained in certain fields, but in most of the fields there was not enough disease to cause an appreciable loss in seed production. In 1932, with favorable environmental conditions prevailing until early summer, the disease occurred much more widely and in a severer form than in 1931. While no data are available on the actual loss sustained, a conservative estimate showed that the loss in seed yield was as much as 25 percent in certain plantings.

In 1933, an appreciable reduction in seed yield was observed in only one planting and this was estimated at less than 5 percent.

In addition to the actual destruction of the floral parts of the plant, with a corresponding reduction in seed yield, the disease through its attack on the leaves, pedicels, and stems often so weakens the plant that many of the seeds (mericarps) produced are undersized and are lost in the cleaning process.

The occurrence of the disease in root beds has not caused appreciable damage therein, but infection on the lower leaves results in the disease being transmitted from the root beds to the seed fields, where serious losses in seed yield may result. Likewise, no appreciable damage has resulted from the occurrence of the disease in the few market-garden plantings observed in California.

FIGURE 6.—Incipient lesions on young carrot leaflet showing the irregular chlorotic areas with irregular brown necrotic centers. ×12.

THE CAUSAL ORGANISM

ISOLATION

Repeated isolations from necrotic leaf spots, dark-brown streaks on pedicels and stems, and from blighted umbellets consistently yielded a similar type of yellow bacterial organism. All isolations were made by immersing diseased material in a mercuric chloride solution,

1:1000, for 1 to 2 minutes, washing thoroughly in sterile water, and cutting into small sections in a drop of sterile water on a flamed slide. Microscopic examination showed bacteria, in great abundance, oozing into the water from the cut surfaces of the tissue, and dilution plates of potato-dextrose agar were poured from suspensions prepared in this way. Dilution plates were also poured from the gummy exudate sometimes found at the base of severely diseased umbels and along the petiole and stem lesions. In 36 to 48 hours, an even and usually heavy seeding of similar circular, light-yellow bacterial colonies appeared on the surface of the poured plates.

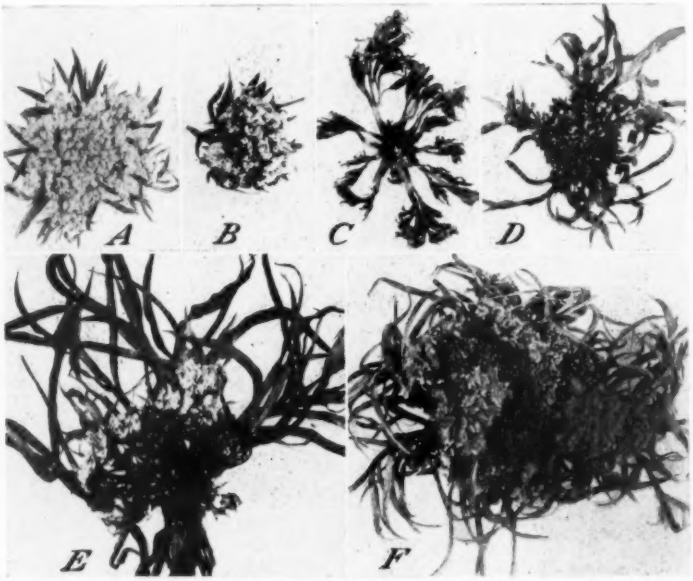

FIGURE 7.—Carrot umbels showing different stages of bacterial blight: *A*, healthy umbel; *B*, young umbel showing scattered dark-colored infected umbellets among the white healthy umbellets; *C*, young umbel showing the death of the involucral bracts and the umbel itself before floral development; *D*, later stage showing all but a few umbellets killed and the few resulting mature ovaries (schizocarps) along one side; *E*, severe one-sided attack of the disease resulting in the death of the involucral bracts and umbellets on that side; *F*, umbel showing irregular killing of the umbellets and scattered patches of apparently normal mature seed.

MORPHOLOGY

The organism is rod-shaped with rounded ends and occurs singly or in pairs (fig. 8, *A*). Single rods were 1.38μ to 2.75μ long by 0.42μ to 0.85μ wide (average 1.95μ by 0.59μ) when taken from a 24-hour potato-dextrose agar slant culture and stained with Ziehl's carbol fuchsin. The organism is motile by means of 1 or 2 polar flagella attached at one end (fig. 8, *B*). Flagella measure up to 7μ in length, as stained by Plimmer and Paine's method (*6*).[3] No spores are formed and capsules have not been demonstrated. The organism is Gram-negative and stains readily with the ordinary bacteriological stains.

[3] Reference is made by number (italic) to Literature Cited, p. 509.

CULTURAL CHARACTERS

The organism grows luxuriantly on neutral (pH 7) potato agar to which 1 percent dextrose has been added. Slightly less rapid growth takes place on neutral beef-extract agar. In a critical study of the growth characters of the organism, all culture media, except where specifically stated otherwise, were adjusted to approximately pH 7 as evidenced by the grass-green color of the medium when the sulphonthalein indicator, brom thymol blue, was added. Inoculations were made by using a 2-mm loop of a water suspension of the organism made by removing the growth from a 24-hour-old agar slant culture and adding to a 10-cc water blank. Two original isolations, a reisolation from an inoculated plant, and a culture of *Bacillus coli* were grown in parallel duplicate series on all culture media. After inoculation, the cultures were held at room temperature (20° to 27° C.).

AGAR POURED PLATES.—On poured plates of potato-dextrose and beef-extract agar, colony growth was faintly visible in 24 hours, and in 48 hours colonies were one-fourth to one-half mm in diameter on the former and slightly smaller on the latter. Surface colonies reached their maximum growth in 4 to 6 days and were 1 to 3 mm in diameter on potato-dextrose agar but somewhat less on nutrient agar. While growth was plainly less vigorous on nutrient agar than on potato-dextrose agar, the character of the growth was the same. Surface colonies were circular, convex or pulvinate, smooth, glistening, and light yellow in color with margin entire. Submerged colonies were small and lens-shaped. The medium was unchanged in color and there was no odor.

AGAR STABS.—Stab cultures on nutrient agar showed best growth on the surface. In 2 weeks, a circular, slightly raised, amber-yellow colony 4 to 5 mm in diameter had developed on the surface around the stab and a slight filiform growth had taken place along the upper half of the stab.

AGAR SLANTS.—On potato-dextrose agar slants, growth was abundant, slightly raised, smooth, glistening, and straw yellow[4] to massicot yellow in color with margin entire. Growth on beef-extract agar slants was similar, although slightly less abundant, and wax yellow to amber in color. There was no change in either medium.

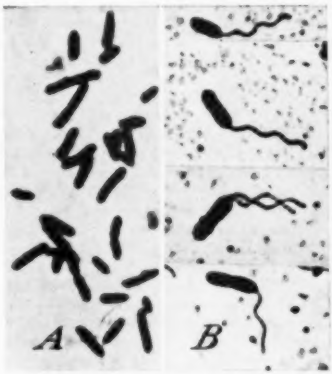

FIGURE 8.—Bacteria causing carrot leaf spot and umbel blight: *A*, Normal cells stained with carbol fuchsin, ×2000; *B*, cells showing polar flagella; stained by Plimmer and Paine's method, ×2000.

GELATIN PLATES.—Nutrient gelatin plates held at 20° C. showed minute surface colonies in 48 hours, but no liquefaction. In 72 hours, saucer-shaped zones of liquefaction were present under the small circular colonies, and in 96 hours the plates were completely liquefied.

GELATIN STABS.—Stab cultures in nutrient gelatin held at 20° C. liquefied slowly. Liquefaction at the end of 7 days was infundibuliform, later becoming stratiform, and was complete in 2 months, with a heavy, yellow flocculent precipitate in the bottom of the tubes.

POTATO CYLINDERS.—Growth on steamed potato cylinders was moderote, flat, spreading, moist, glistening, light lemon-yellow in color, and covered the entire slant in 5 days. In 10 days the upper portion of the cylinder was noticeably darker than the control.

MILK.—There was no apparent change in milk until the seventh day, when the milk was cleared without coagulation. The cleared liquid was a light-straw to amber color with light-yellow precipitate in the bottom of the tube.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

LITMUS MILK.—Litmus milk was cleared in 7 days, and in 13 days there was a small band of light-purple liquid on the surface while the remainder of the liquid was a dark-amber color.

METHYLENE BLUE IN MILK.—In 3 days the color started to fade from a bright green to a dull blue, and in 7 days had disappeared from all except the top layer of the medium. In 13 days the medium was entirely cleared and the liquid was amber colored, but at the end of 21 days the liquid had become a light blue-green color which turned a darker blue when the tubes were shaken.

BROM CRESOL PURPLE IN MILK.—In 7 days the medium was entirely cleared and the liquid was a dark-pink to purple color. In 21 days, the liquid was a reddish purple, showing that there was no acid produced in milk.

REDUCTION OF NITRATES.—In fermentation tubes containing a 1-percent Difco peptone solution and 1 percent potassium nitrate, there was strong clouding in the open arm, but no visible growth in the closed arm and no gas was formed. Test-tube cultures in nutrient broth containing 1 percent potassium nitrate showed no reduction of nitrates when tested with the sulphanilic acid and naphthylamine acetate solution.

CARBON METABOLISM.—To test for gas production, 1 percent of each of the following carbon compounds was added to a 1-percent solution of Difco peptone, dextrose, sucrose, lactose, maltose, mannite, and glycerin, and placed in ordinary inverted fermentation tubes. With all the carbon sources there was good growth in the open arm but none in the closed arm and no gas was formed.

In order to determine whether acid was produced from carbon compounds, four series of media were prepared and the indicator brom thymol blue was added in a concentration of 0.0016 percent, which produced a grass-green color when the medium was adjusted to pH 7. Beef-extract broth was made up according to the recommendations of the Society of American Bacteriologists (*9*) and divided into two portions, to one of which was added 1.5 percent agar. The two solutions were then neutralized with normal sodium hydroxide solution and brom thymol blue was added. Two similar solutions of synthetic medium (*9*) were prepared and the indicator added. Thus, four basal media were available, each of which was divided into 13 portions to which was added 1 percent of each of the following 13 compounds: Dextrose, sucrose, maltose, lactose, mannite, l-arabinose, d-galactose, d-mannose, rhamnose, raffinose, xylose, trehalose, and glycerin. These media were tubed and sterilized at 7 pounds pressure for 45 minutes.

A second series of similar basal media were prepared and autoclaved. The 13 carbon sources were made up in concentrated solutions and filtered through sterile Berkefeld N filters. The filtrates were then added aseptically to the sterile basal medium in sufficient quantity to give a concentration of 1 percent of the carbon source in the medium. These media were then tubed aseptically. This resulted in a series of media in which the carbon compounds had been autoclaved at 7 pounds' pressure for 45 minutes and a series in which they had not been subjected to heating.

After 5 days' incubation to insure sterility of the media, inoculations were made in the usual way. The slightest acid production was evidenced by a faint fading of the green color to yellow, and strong acid was shown by a quick change from green to yellow. Three strains of the organism were used in duplicate cultures, and, with a few exceptions, the six cultures on each medium were identical. The results of these tests from notes taken 14 days after inoculation are presented in table 1.

From these results, it is apparent that the organism produces acid when grown on the following carbon compounds: Dextrose, lactose, d-galactose, sucrose, d-mannitol, xylose, trehalose, d-mannose, l-arabinose, raffinose, and glycerin. Acid was not produced on maltose and rhamnose, both of which showed a change to alkalinity in the beef-extract media. Except for the acid production with the heated trehalose and d-mannose in the beef-extract media and the unheated xylose and d-mannitol in the synthetic media, there was not much difference in reaction between the heated and unheated carbon compounds. In general, the synthetic medium favored acid production.

AGAR WITH SUGARS.—As a further test for acid production, the organism was grown on beef-extract agar containing 1 percent of the following carbon compounds: Dextrose, lactose, maltose, and sucrose, and enough litmus to produce a lavender color. There was no indication of acid production in the dextrose, lactose, and maltose agars, but there was a slight acid production in the sucrose agar as evidenced by a pale-pink color of the medium. The dextrose and sucrose media became pale yellow in 7 days, but after 21 days the original color had returned.

TABLE 1.—*Summary of acid production*[1] *by Pseudomonas carotae from carbon compounds in 4 basal media prepared with and without heat sterilization*

Carbon source	Method of sterilization	Type of medium			
		Beef extract		Synthetic	
		Broth	Agar	Broth	Agar
Dextrose	Heat	−	−	++++	+++
	Filtration	−	−	+++	++++
Maltose	Heat	−−	−	−	−
	Filtration	−−	−−	−	−
Lactose	Heat	−	−	++	++
	Filtration	−−	−−	++	++
d-Galactose	Heat	++	−	+++	++++
	Filtration	++	−	++++	++++
Sucrose	Heat	++	+	+++	[2]−++++−
	Filtration	+	+	+++	+++
d-Mannitol	Heat	−−	−	−	−
	Filtration	−−	−−	++	+++
Xylose	Heat	−−	−−	−	++
	Filtration	−−	−−	++	++
Trehalose	Heat	+	+	++	+++
	Filtration	−	−	+++	+++
d-Mannose	Heat	+	−	++++	+++
	Filtration	−	−	+++	+++
l-Arabinose	Heat	−−	+++	−	+
	Filtration	−−	++	−	+
Rhamnose	Heat	−−	−−	−	−
	Filtration	−−	−−	−	−
Raffinose	Heat	−	−		+
	Filtration	−	−	+	++
Glycerin	Heat	−	−	+	++
	Filtration	−−	−	++	+++

[1] ++++=Strong acid as evidenced by change of brom thymol blue from green to yellow; +++=medium acid; ++=weak acid; +=faint acid; −=no color change; −−=alkaline as indicated by change from green to blue.

[2] 2 strains showed no change and 1 showed strong acid.

ACTION ON STARCH.—There was no diastatic action on starch when the organism was grown on plates of beef-extract agar containing soluble starch and tested by flooding with potassium iodide-iodine solution.

INDOL PRODUCTION.—Cultures in beef-extract broth gave no test for indol with Salkowski's reagent.

COHN'S SOLUTION.—No growth.

FERMI'S SOLUTION.—No growth.

USCHINSKY'S SOLUTION.—Moderate growth, but no pigment formed.

TOLERATION OF SODIUM CHLORIDE.—Cultures in beef-extract broth containing 2, 3, 4, 5, and 6 percent sodium chloride showed that the organism was able to tolerate concentrations as high as 4 percent.

ACID TOLERATION.—A series of beef-extract broth cultures was adjusted colorometrically to a range of pH values by the addition of N/20 HCl. These media were tested electrometrically and found to have the following pH values: 4.45, 4.50, 4.60, 5.10, 5.40, 5.60, 5.80, and 6.20. No growth occurred in tubes of pH 5.10 or below, but fair growth occurred in tubes of pH 5.40 and moderate to strong growth in tubes of pH 5.60 to 6.20.

TEMPERATURE RELATIONS

Potato-dextrose-agar plate and slant cultures were placed in moist chambers and incubated at the following temperatures: 5°, 9°, 13°, 17°, 21°, 25°, and 30° C. No growth occurred at 5° and 9° and only slight growth at 13° after 9 days' incubation. Growth was somewhat delayed at 18° and 21°, but after 9 days' growth at 21° was moderate and only slightly less than at 25°. Growth at 25° was vigorous, but slightly less so than at 30° the first 3 days. After 9 days there was no appreciable difference in the amount of growth at 25° and 30°. While the organism grows well at a temperature as low as 18°, maximum growth occurred at 25° to 30°.

The thermal death point was determined by subjecting a dense water suspension (pH 7) of the organism in small thin-walled vial test tubes to 10 minutes' exposure in a water bath and recording the temperature inside of a similar tube of water. The temperature was not allowed to vary more than 0.2° during each test. Dilution plates were poured and it was found that the thermal death point was 49° C.

To determine the ability of the organism to withstand freezing, dense water suspensions were placed aseptically in small sterile vials in cold storage at −10° C. Freezing occurred in a few hours. Dilution plates poured from the original suspension before freezing showed approximately 45,000,000 bacteria per cubic centimeter. After the suspension had been frozen 1 month there were approximately 11,000,000 per cubic centimeter, and after it had been frozen 2 months there were approximately 587,600. These results show the bacteria to be rather resistant to freezing.

DESICCATION

Tests made by dropping sterile cover glasses, on which a drop of a water suspension of the organism had been allowed to dry, into beef-peptone broth showed that the organism was able to withstand 204 hours of drying on glass.

TAXONOMY

The organism causing carrot bacterial blight apparently differs from any that has previously been described. While several bacterial species have been reported as pathogenic on carrot, they all differ in pathogenicity as well as morphologically and physiologically from the organism discussed herein.

Potter (*7*) in 1899 reported a white, polar flagellate organism as the cause of a white soft rot of turnips, named it *Bacterium destructans* and listed *Daucus carota* as a host. Later, in 1909, Harding and Morse (*3*), in examining cultures of Potter's *Bact. destructans*, determined it to be peritrichiate and not essentially different from *Bacillus carotovorus*, which Jones (*4*) described in 1901 as the cause of a soft rot of vegetables. Smith in 1920 (*8*) substantiated Harding and Morse's findings. In 1915 Takimoto (*10*) described a white polar flagellate organism, *Bact. viciae*, as the cause of leaf and stem lesions on *Brassica rapa*, *Daucus carota*, *Ipomoea batatas*, and *Vicia faba*. A comparison of the morphological and cultural characters of *Bact. viciae* with those of the organism causing bacterial blight of carrot shows the two to be entirely distinct species. S. G. Jones (*5*) in 1922 found a white polar flagellate organism causing a white rot of turnip, radish, carrot, and cabbage in Wales and concluded that it was probably a varietal form of *Pseudomonas destructans* (Potter).

Since the organism known to be the cause of carrot bacterial blight is apparently an undescribed species, it is given the following brief characterization.

TECHNICAL DESCRIPTION

Pseudomonas carotae, n. sp.[5]

Cylindrical rods, rounded at ends, single or in pairs; single cells 1.38μ to 2.75μ by 0.42μ to 0.85μ (average 1.95μ by 0.59μ); motile by 1 to 2 (usually 1) polar flagella; aerobic, nonspore-forming; no pronounced capsulation; involution forms not observed. Stains readily with carbol fuchsin and gentian violet; Gram-negative.

[5] Migula's classification, Buchanan's revision (*2, p. 45*), and the revision adopted by the committee of the Society of American Bacteriologists (*11*). According to Bergey et al. (*1*) the combination would be *Phytomonas carotae* n. sp.

Surface colonies on potato-dextrose agar round, smooth, convex or pulvinate, glistening, margin entire, straw yellow to massicot yellow. Gelatin liquefied; milk cleared in 7 days with no coagulation or acid production; nitrates not reduced; no indol; no gas with dextrose, saccharose, lactose, maltose, d-mannitol, or glycerin; acid produced from dextrose, lactose, d-galactose, sucrose, d-mannitol, xylose, trehalose, d-mannose, l-arabinose, raffinose, and glycerin; no acid from maltose or rhamnose. No diastatic action on starch. No growth in beef-extract broth at pH 5.10; fair growth at pH 5.40, and strong growth at pH 5.60. Tolerates 4 percent sodium chloride beef-extract broth at pH 7. No growth in Cohn's or Fermi's solutions; moderate growth in Uschinsky's solution, without pigment formation. Grows at 13° to 30° C.; optimum between 25° and 30°; thermal death point 49°; resistant to freezing in water; relatively resistant to drying on glass.

Pathogenic on leaves, stems, and floral parts of *Daucus carota* L. var. *sativa* DC.

Type locality: Yolo and Sacramento Counties, Calif.

INOCULATION TESTS AND LENGTH OF INCUBATION PERIOD

Typical leaf lesions have been repeatedly produced by spraying plants of all ages with a water suspension made from a 48-hour-old agar culture of the causal organism and holding them in an inoculating chamber from 10 days to 2 weeks at Davis, Calif. Plants similarly sprayed and kept in the open or in an inoculating chamber at Berkeley, Calif., likewise showed the first evidence of infection in 10 to 12 days after inoculation.

On April 9, 1932, a large carrot plant growing in the greenhouse and developing flower umbels, was sprayed with a water suspension of bacteria and covered with a large manila bag. Infection did not show until 10 days after inoculation, and on April 25, 6 of the umbels were killed and 4 others showed typical necrotic spots on the involucral bracts. Seventeen young umbels which developed after the plant was inoculated were free from the disease. On May 6, 1932, the lower older leaves were removed from 18 young plants growing in a row of carrots in the greenhouse and the younger leaves sprayed with a water suspension of the bacterial culture used in the foregoing inoculation. Large flowerpots were inverted over the plants for 3 days to form moist chambers. The plants were observed frequently for evidence of infection, but it was not until May 31, 25 days after inoculation, that typical leaf spotting was noted on the leaves of all of the 18 plants. The disease developed slowly, and on June 18, 1 plant showed typical dark-brown streaks on a leaf petiole. Fourteen control plants similarly treated with sterile water remained healthy. The organism was recovered from the lesions on the inoculated plants. During this trial the temperature was relatively high and the humidity low in the greenhouse. Five large carrot plants growing in a garden were thoroughly sprayed with a water suspension of the carrot-blight organism on May 6, but no disease resulted.

On June 13, 1932, 10 young carrot plants growing in pots were sprayed with a water suspension of another culture of the bacteria and placed in a large inoculating chamber. Five plants sprayed with sterile water were placed in the same chamber as controls. The inoculating chamber had a galvanized iron roof and heavy canvas sides. High humidity and relatively low temperature were maintained inside the chamber by directing a fine spray of water on the peak of the roof, and air circulation was maintained by an electric fan inside the chamber. In 10 days considerable necrotic spotting was evident on the leaf segments. The plants were removed from

the chamber at the end of 10 days, and were observed until July 11, but very little additional spotting appeared. The control plant shows no evidence of infection.

A duplicate set of inoculations, consisting of 8 young carrot plants in pots, were made on November 11, 1932. The plants were sprayed with a water suspension of the bacteria, and 8 were placed in the above-described chamber in a greenhouse where no steam heat was being used and the other 8 were placed in a glass inoculating chamber in a greenhouse where the temperature was maintained at 65° to 70° F. In neither case was a water spray used to maintain humid conditions. In 11 days a few incipient lesions were evident on all plants in both chambers. The plants were removed from the chambers to a greenhouse bench and at the end of 1 month typical bacterial lesions were present on many of the tips and on the margin of many leaf segments (fig. 2, *A* to *G*). The spotting was so severe on some leaves that they were dying. Control plants, sprayed with sterile water and held in the same chambers remained healthy.

Four young carrot plants treated as above on December 19 and held in the glass chamber showed the first evidence of infection on December 30, and by January 3 there were many well-defined necrotic spots on the young leaflets and segment tips, with the characteristic chlorotic area around the dead spots. On March 22, 1933, a large carrot plant showing several developing umbels was thoroughly sprayed with a water suspension of the bacteria and placed in a moist chamber. Twelve days after inoculation, incipient infection was evident on the segment tips, and approximately 40 percent of the umbellets in the terminal umbel were dark brown and had a water-soaked appearance.

In all the above-described inoculations, microscopic examinations were made to determine the presence of bacteria in the infected areas, and in many cases reisolations were made and the pathogenicity of the organism proved by inoculation trials.

On October 8, 1932, O. C. Whipple inoculated five pots of young carrot plants in the greenhouse at Berkeley by spraying them with a water suspension of the bacteria, and in 11 days numerous spots were evident on the leaves (figs. 3 and 6.) Thirty-eight days after the plants were inoculated, 48 of the 51 plants showed foliage infection. Five pots of plants sprayed with sterile water showed no evidence of the disease. Another series of 13 pots of young carrot plants were similarly inoculated by Whipple on November 3, 1932, and 4 pots of plants were sprayed with sterile water as controls. The first evidence of infection was visible as translucent chlorotic dots on the small leaflets 12 days after the plants were sprayed with the bacterial suspension (fig. 1, *A*). Twenty-seven days after inoculation, 62 of the 71 plants showed abundant leaf spotting, and the 21 plants in the 4 control pots were all healthy. A third series of inoculations were made by Whipple on November 5, 1932, consisting of 6 pots of young carrot plants and 4 pots of controls. Again the first evidence of infection was visible 11 days after inoculation.

In all the inoculation trials conducted at Davis and Berkeley, no evidence of infection was visible in less than 10 days after the plants were sprayed with a water suspension of the organism, and the usual incubation period was from 10 to 12 days.

SOIL AND SEED AS POSSIBLE SOURCES OF INFECTION

The presence of the disease in the umbels insures seed (mericarp) contamination, and observational evidence indicates that the seed is one of the primary sources of infection. In early December 1932, a survey of three carrot-root beds planted from seed near Sacramento revealed the presence of the disease in each. The plants were then of considerable size and some were being dug for transplanting to the seed fields. There was abundant leaf spotting on the lower, older leaves, some of which had been killed by the disease, and spotting was evident on the upper, younger leaves. In one of the root-bed plantings, an estimate of 25 to 40 percent of the plants showed the disease, and in another large root bed from 40 to 90 percent of the plants showed the disease, the amount of disease varying in different portions of the fields.

One planting of 25 acres from seed which, according to information available, had not been previously planted to carrots, showed severe necrotic spotting of the older leaves. In this field the disease, while quite generally distributed, was much worse in certain spots, especially in the low areas. Adjoining this field was a later planting of carrots which had developed only a few true leaves. Scattering cases of typical leaf spots were observed on the lower first leaves. On December 9, typical necrotic spotting was observed on the lower leaves of young carrots in a commercial planting for market purposes near Sacramento. The age of the carrots was not determined, but the roots were one-half to 1 inch in diameter.

From each of the foregoing plantings successful isolations were made and the pathogenicity of the organisms secured was demonstrated by greenhouse inoculation trials.

On November 24, 1933, carrot-root beds near Sacramento were again inspected for the presence of the disease. In a small 1-acre plot of very young plants, several typical cases of the disease were observed on the first foliage leaves (fig. 4, *A*). Only a small number of infected plants was found; in some cases only a single plant showed the disease while in a few cases several adjacent plants showed typical leaf lesions. Near this planting, but on lower ground, there was approximately 10 acres of larger carrots, some of which were almost ready to be transplanted to the seed fields, and considerable leaf spotting was found on the lower leaves. As in 1932, the disease appeared worse in certain areas, but there was very little evidence of spotting on the upper leaves. Practically no rain had fallen since the carrots were planted, and this, combined with the long incubation period of the organism, no doubt caused the disease to be confined largely to the lower leaves. Carrots had been grown in both these fields in 1932, and during that year had shown a rather general, and in some spots, severe infestation.

In the same section there was a 15-acre planting of carrots of approximately the same age as those in the 10-acre field, but on much higher ground, which precluded any possibility of soil drainage from the latter field. This field was known not to have been in carrots in 1932. While the disease was found in this planting on some of the lower leaves, it was not so severe as in the field where diseased carrots had been grown in 1932. One typical severely diseased plant was observed, showing death of the lower leaves and severe necrotic spot-

ting of the upper leaves. The plants surrounding it showed less severe leaf spotting, indicating secondary spread from the severely diseased plant.

Field evidence thus points to both soil and seed as possible sources of infection. In view of this evidence, it is advisable to secure seed from disease-free fields and locate root beds on soil where carrots have not been grown the previous season.

During the season of 1932, seed umbels which showed varying degrees of destruction from this disease and which had matured a partial crop of seed, were hand-picked and crushed in a bag and the mature seeds (mericarps) were removed from the trash by a screen. On December 21, 1932, eight flats of steam-sterilized soil were planted with this seed. The plants came up normally, and a careful examination was made at frequent intervals from the time they came through the soil until the experiment was discontinued on April 17, 1933. Approximately 1,200 plants were produced in the 8 flats and there was no evidence of the disease at any time.

A second trial was started on February 21, 1933, by planting twenty-eight 6-inch pots of sterile soil with seed from the same lot mentioned above. The pots were placed in the open on the north side of a greenhouse and were sprinkled frequently. As in the previous trial, germination was normal, and the plants were carefully examined at frequent intervals for evidence of the disease. On May 8, the final examination was made and the plants pulled. A total of 2,473 plants were produced in this trial and no evidence of the disease appeared at any time.

While the foregoing trials gave only negative evidence as to seed transmission of the disease, it is felt that further tests should be made before definite conclusions are drawn, for field evidence strongly points to the seed as a possible source of the disease.

In early November 1933, a small quantity of carrot seed from a field in which bacterial blight had not occurred, was immersed momentarily in a water suspension of an agar culture of the bacterial blight organism. This seed was then placed in sterile glass dishes and allowed to dry. On November 13, four flats of steam-sterilized soil were planted with this seed and two flats of soil with uninoculated seed. The flats were placed in the open and were subjected to very moist and rather cool climatic conditions during December 1933 and January 1934, with the result that growth was less vigorous than normally occurs in the root beds.

The young plants were examined at frequent intervals for evidence of the disease, and on January 30 six young plants were found with typical lesions on the lower first leaves (fig. 4, *B* and *C*). Isolations and subsequent inoculations were made and the identity of the organism was proved. Following the first appearance of the disease many other plants showed similar symptoms, always on the lower first leaves. On February 14, the plants in definite areas in the flats were pulled and examined for the presence of the disease. From these data, it was estimated that there were approximately 4,500 young plants in the 4 flats and that from 5 to 7 percent showed typical lesions on the lower first leaves. There were approximately 2,500 plants in the 2 flats planted with clean seed and none showed evidence of the disease.

SUMMARY

A bacterial leaf spot and umbel blight of carrots occurred in 1931 to 1933 in the root beds and seed fields of the extensive seed-producing area of the upper delta region of the Sacramento Valley, Calif.

The disease attacks the leaves, petioles, peduncles, stems, and umbels and is characterized by irregular necrotic spots on the leaves and involucral bracts, dark-brown streaks on the petioles, peduncles, and stems, and a blighting of a portion or all the flowers in an umbel.

The occurrence of the disease on the leaves may weaken the plants, but the main damage results from the destruction of the seed umbels with a consequent reduction in seed yield.

The causal organism is easily isolated from diseased tissue, forms yellow colonies, and is motile by polar flagella. Gelatin is liquefied; milk cleared in 7 days without coagulation; nitrates not reduced; acid produced from dextrose, lactose, d-galactose, sucrose, d-mannitol-xylose, trehalose, d-mannose, l-arabinose, raffinose, and glycerin, but not from maltose and rhamnose. The optimum growth occurs between 25° and 30° C. The thermal death point is 49° C. The organism is resistant to freezing.

In inoculation tests infection has been obtained by spraying a water suspension of the organism on the leaves and flower umbels of carrot plants and holding them in an inoculating chamber 10 to 12 days. In no case has the incubation period been less than 10 days.

The causal organism, apparently an undescribed species, is briefly characterized and named *Pseudomonas carotae* n. sp.

Soil infestation may be a source of infection, since the disease has been found in root-bed plantings from seed on soil in which a diseased crop was grown the previous year.

The prevalence of floral infection insures seed contamination, and observational evidence in the root beds suggested the seed as the primary source of the disease. During the winter of 1932–33, 3,673 plants from hand-selected seed from diseased plants were grown in two seed tests in steam-sterilized soil, but no evidence was obtained that the causal organism was harbored by the seed.

During the winter of 1933–34, approximately 4,500 plants were grown from seed which had been immersed in a water suspension of the causal organism and dried before they were planted, and 5 to 7 percent of the plants showed typical bacterial lesions on the lower first leaves.

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PECAN ROOT GROWTH AND DEVELOPMENT[1]

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INTRODUCTION

The relation of the root system of pecan trees, *Hicoria pecan* Britt., to nursery and orchard practices has been studied at the Georgia Agricultural Experiment Station for several years.[2] The relation of mycorrhizal infection to the growth of pecan roots has also been studied.[3] The present paper deals with certain morphological, physiological, and anatomical characters of pecan roots as they relate to growth and development.

Data are also being obtained on the most suitable depth of setting trees, distance between, depth of cultivation, season of cultivation, and methods of fertilizing and cover-cropping orchards.

The gross morphology of pecan roots was studied by digging the roots and charting their position on coordinate paper as they were being dug. Some were dug with the specific purpose of studying the tap root, others were dug to examine lateral roots, and still others to examine the fibrous roots. Some trees were grown under controlled conditions and the roots were examined before they were a year old; some were dug from nurseries, and others from orchards that had been bearing for many years.

An attempt was made to dig trees that had been grown on the prevailing soil types found in Georgia, as well as in different locations with respect to water table and drainage. Likewise, artificial soils were prepared and roots were grown in them, the roots being removed later for examination.

Studies on the development of new roots were made by giving specific treatment to a part or all of a tree or root before it was planted; portions were then dug periodically for examination. Studies of growth rate were made by growing seedlings in boxes with glass sides. When the boxes were inclined the roots grew close to the glass on the inside and could be measured accurately, and as often as desired. The glass was fitted with heavy oilcloth on the outside to keep out the light, and most measurements were made at night.

THE SPREAD OF PECAN ROOTS IN THE SOIL

The crowding of pecan roots between adjacent trees begins much earlier than the crowding of branches of the same trees. Figures 1 and 2 show the relation of height and spread of branches to depth and spread of roots. This subject was discussed at length in a previous publication.[4]

[1] Received for publication Sept. 22, 1933; issued October 1934. Paper no. 39, Journal Series of the Georgia Agricultural Experiment Station.

[2] WOODROOF, J. G. THE RELATION OF THE ROOT SYSTEM OF PECAN TREES TO NURSERY AND ORCHARD PRACTICES. Ga. Expt. Sta. Bull. 176, 15 pp., illus. 1933.

[3] WOODROOF, N. C. PECAN MYCORRHIZAS. Ga. Expt. Sta. Bull. 178, 26 pp., illus. 1933.

[4] WOODROOF, J. G. See footnote 2.

The first year the top branches very little and grows to a height of about 1 foot. The taproots grow from 1 to 3 feet, depending on the soil fertility, moisture, and texture. Branching of the seedling taproot extends throughout its length, but often a high percentage of these small laterals die during the fall of the first year. The second year the distribution of growth is very similar to that of the first, considerable branching occurring in both top and roots. The taproot reaches a depth of about 4 feet, and the laterals make considerable growth in the horizontal direction. Typical mycorrhizal roots are usually formed first during the second year.

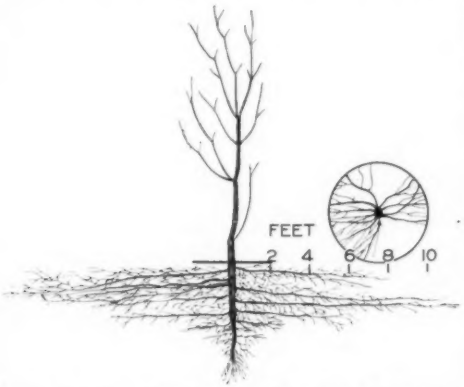

FIGURE 1.—A 6-year-old pecan tree with a spread of branches 5½ feet, a height of 13 feet, a root spread of 24 feet, and a root depth of 6 feet. Trees of this size are frequently transplanted into an orchard, in which case the roots are cut back to a spread of about 1 foot. In the circle is shown the orientation of the 11 largest lateral roots.

During the third year the height of tree, depth of taproot, and spread of laterals are about equal, but the branch spread is considerably less. This is the age at which most trees are transferred from the nursery to the orchard. In the fourth year the height of tree exceeds the depth of roots for the first time, and the spread of the roots is about double that of the branches.

Little additional deepening of the roots occurs after 5 years, but the tree continues to grow in all other directions. By the time a tree is 10

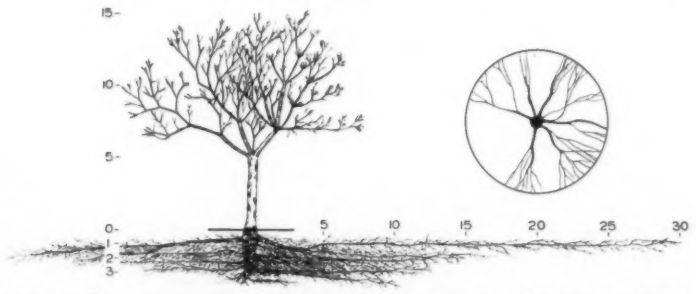

FIGURE 2.—A 12-year-old Success pecan tree growing on a sandy loam topsoil with a sandy clay subsoil. The tree had a spread of branches of 17 feet, a height of 15 feet, a total spread of roots of 47 feet, and root depth of 4½ feet. In the circle is shown the orientation of the six largest lateral roots.

years old the height of the top is about four times the depth of the taproot, and the spread of the branches is about one-half the spread of the roots. Branching of both stems and roots is more pronounced as the tree grows older, and at 20 years or more the tree is made up almost entirely of branches both above and below ground.

Pecan roots tend to grow most profusely in those areas or strata of soil in which conditions are most favorable for growth. In a cultivated orchard the region in which roots are most dense is usually from 12 to 18 inches below the soil surface. Deep cultivation tends to force the roots to lower levels, while shallow cultivation, a high water table, or mulch with litter or cover crops allows the roots to grow near the surface. Fibrous roots tend to grow toward the soil surface until they are hindered in doing so by drought, freezes, cultivation, or other deterrents.

Pecan roots may be divided into the following classes: (1) Taproots, which grow straight down and determine the depth of penetration of the roots under the particular conditions; (2) lateral roots, which usually grow in a horizontal direction straight from the tree at a depth of from 1 to 2 feet, and determine the lateral spread of roots under the existing conditions; (3) fibrous roots, which grow in all directions from the laterals. Fibrous roots are subject to constant dying and replacement as a result of varying growing conditions; mycorrhizal roots, which are a type of fibrous roots and grow in dense masses throughout the entire area in which roots are growing.

ROOT GROWTH UNDER VARIOUS ENVIRONMENTAL CONDITIONS

With abundant moisture and the approach of warm weather in the spring, roots of all orders make vigorous growth with moderate branching. Roots sometimes grow 10 cm without branching, then produce 4 to 6 branches while making 5 to 10 cm more growth. The branches run in different directions, each of them growing from 4 to 10 cm, then beginning to branch. As the season advances growth is less vigorous and branching more profuse.

The spring growth of roots begins a little in advance of the spring growth in shoots. Roots are more sensitive to adverse conditions either of the soil or weather but are also more responsive to the return of favorable conditions. Therefore roots usually make from 4 to 8 cycles of growth during the year.

EFFECTS OF MOISTURE, LIGHT, AND SOIL TEXTURE

Table 1 shows the relation of root order and root diameter to daily growth and represents the results of more than 1,700 measurements. These were made on young seedlings only, but an approximate relationship between size of root and rate of growth appears to hold for all roots.

Roots are very efficient in making use of the moisture supplied to the soil by a relatively light summer rain. They also respond to the heat supplied by the sun during the first warm days of spring several weeks before there is any response in growth in the shoots.

Eighteen taproots of young seedlings exposed to reflected sunlight for 2 weeks had an average daily growth of 9.5 mm. The same roots when kept in total darkness for a week had an average daily growth of 8.5 mm. The same roots were killed by direct exposure to the sun during the following week. The seedlings were growing in a tall box with a glass side, and the soil moisture was kept as constant as possible throughout the period of the experiment.

Figure 3 represents six taproots, drawn to scale, showing typical modifications in form caused by differences in soil texture and moisture content. These roots varied in depth from a little over 3 to 9

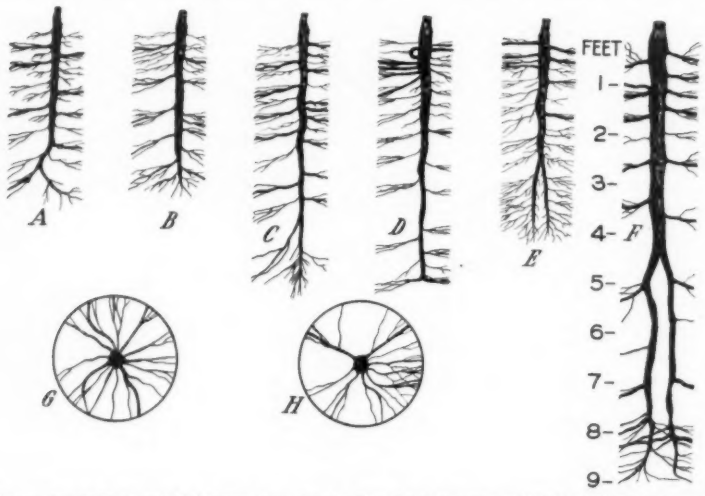

FIGURE 3.—*A–F*, taproots of six pecan trees, showing how their form was modified by different soils. The type of subsoil caused a variation in the length of taproot from a little over 3 feet to more than 9 feet. The length of taproot was not influenced by age of tree (after 3 years) nor the spread of branches. *G*, *H*, orientation of the lateral roots.

feet. When the soil was loose, coarse, or poor in nutrients, or when the water table was low the taproot has been found to penetrate to a depth of 10 or more feet.

TABLE 1.—*Relation of root order and root diameter to the daily growth rate in pecan trees*

Root order	Diameter of roots	Number of roots making indicated growth in millimeters												
		0	1	2	3	4	5	6	7	8	9	10	11	12
	Millimeters													
Taproots	2–3	0	0	1	1	4	15	16	20	30	39	58	40	49
First-order laterals	½	135	198	270	191	191	136	18	8	12	4	2	5	0
Second-order laterals	¼–½	0	50	21	0	0	0	0	0	0	0	0	0	0

Root order	Diameter of roots	Number of roots making indicated growth in millimeters												
		13	14	15	15	16	17	19	20	21	22	23	24	25
	Millimeters													
Taproots	2–3	64	46	33	30	18	15	12	9	3	2	4	0	1
First-order laterals	½	0	0	0	0	0	0	0	0	0	0	0	0	0
Second-order laterals	¼–½	0	0	0	0	0	0	0	0	0	0	0	0	0

Roots that form at right angles directly from the taproot are laterals of the first order; those arising directly from these are laterals of the second order, and so on.

Lateral roots of the first order of 6-week-old seedlings were measured at 48-hour intervals for rate of growth. They had about 12 laterals each, the oldest being about 15 cm long and growing less than 0.2 mm per day. The most vigorous laterals grew from 3 to 9 mm per day when about 6 cm in length. The youngest laterals grew less rapidly. Thus each lateral began growth at less than 0.2 mm per day, rapidly increased to 1.0 mm, and finally to 10 mm on about the tenth day. After 2 weeks, growth was gradually retarded, finally stopping in less than a month. This cycle is apparently followed by

FIGURE 4.—Sinuous growth of pecan roots.

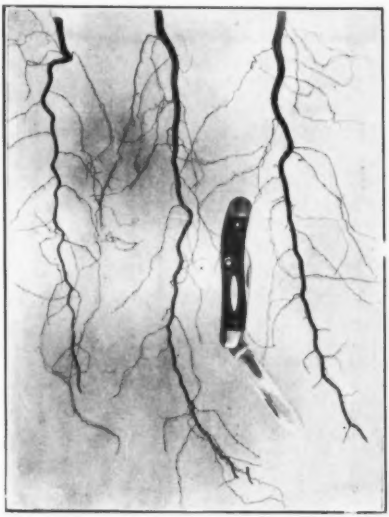

FIGURE 5.—A pecan root that was cut and gave rise to a number of branches; the smallest roots are mycorrhizal.

all laterals of the first order. Those occupying a zone of from 20 to 40 cm back of the tip of the taproot were most active, while those either closer to or further from the tip made less rapid growth.

Lateral roots are at first long and slender, performing the function mainly of absorption. But as they increase in age and size they become organs of storage and support, functions which they maintain throughout their existence. As they in turn produce feeding roots they act as conductors of material to and from the taproot.

While lateral roots tend to grow straight from the taproot, their course is practically never direct. There are always crooks, bends, or twists which give the laterals slack (fig. 4). When lateral roots are caused to turn from a straight course they often produce branches on the convex side.

Lateral roots give rise to hundreds of branches. These grow in every direction, producing thousands of very fine, much-branched fibrous roots that thoroughly permeate the soil. Many are mycorrhizal roots. Figure 5 illustrates the branching of fibrous roots.

EFFECTS OF ATMOSPHERIC AND SOIL TEMPERATURES

The response of roots in dry soil to a renewed moisture supply is remarkably rapid. Following a drought of 2 months, a 0.69-inch rain wet soil in the open field to a depth of about 5 inches. Roots from trees of all ages clearly showed new growth to a depth of the moist

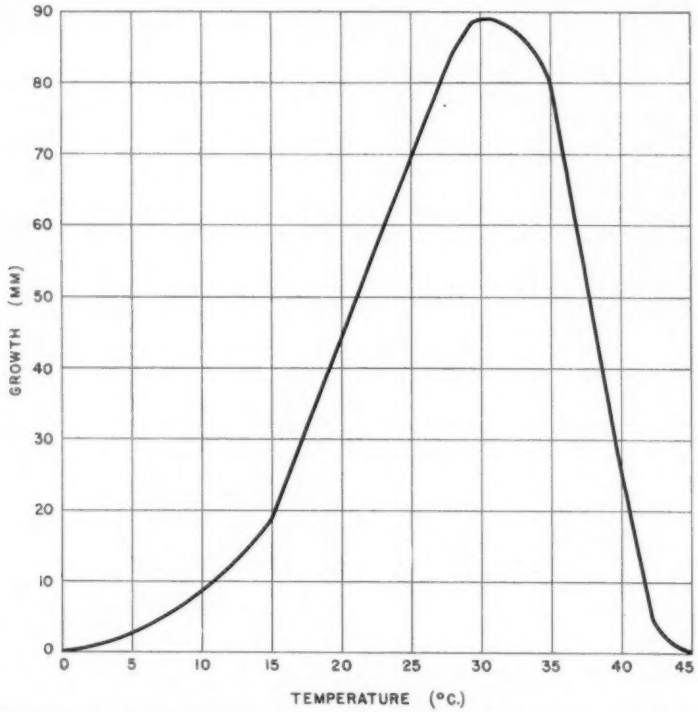

FIGURE 6.—Amount of growth made by 10 taproots when subjected to various temperatures for 24 hours.

soil but none below that point. Roots grew from 2 to 4 mm within 24 hours after the rain. The rain came from the east, causing the soil on the east side of the trees to become wetter than that on the west side. Counts of the growing points per cubic foot of surface soil on the west and east sides of the tree, at the end of the branches, were 1,500 and 32,000 respectively.

Pecan roots were examined immediately after the atmospheric temperature had been −7° C. on three successive nights. The surface soil froze each night but warmed to about 4° at noon. Immediately below the 3 surface inches the soil temperature was maintained at between 4° and 7°. At lower depths the temperature was slightly

higher. After carefully studying the soil depth, soil temperature, and root growth, it was found that no growth was evident in the soil area that had been frozen for three successive nights; a feeble growth of roots occurred in the area that was maintained at from 4° to 7°; and slightly more growth was found in lower areas of soil.

Further study of the effect of temperature on root growth was made by carefully removing soil from an area 1 foot deep, 1 foot wide, and 2 feet long, from beneath both Alley and Frotscher trees, at 15-day intervals for a year. The soil was washed through a screen and the new root growth measured. While there was no close relation between the amount of root growth and the temperature, it was evident that some root growth occurred throughout the entire winter.

The amount of growth made by roots at various temperatures was studied also under controlled conditions. Nuts were germinated in soil. The seedlings were then removed from the soil, measured, and transferred to compost in paraffined, paper-board quart cans. From 4 to 10 seedlings were placed in each can, with soil and a definite amount of moisture. The cans were placed in constant-temperature chambers ranging from −2° to 45° C. Water was added as needed, holes in the bottom allowing the excess to drain out. After 4 days the seedlings were removed from each can and the increase in length of tap roots measured. Forty-two lots were used, including 5 series and 23 different temperatures. The results are shown in figure 6 and table 2.

TABLE 2.—*Influence of temperature on root growth of pecans*

[10 roots observed for 4 days]

Temperature (° C.)	Growth in length	Condition of roots at end of period	Temperature (° C.)	Growth in length	Condition of roots at end o period
	Millimeters			*Millimeters*	
−2	−2.00	Entire roots dead.	23	62.0	Roots uninjured.
−1	.15	Root tips dead.	24	69.0	Do.
1	1.4	Roots uninjured.	27	81.1	Do.
3	5.2	Do.	30	88.1	Do.
6	4.6	Do.	33	73.1	Do.
9	7.6	Do.	34.5	70.7	Do.
12	11.3	Do.	36	57.0	Do.
13	13.1	Do.	38	37.8	25 percent of tips dead.
14	14.3	Do.	41.5	10.0	All root tips dead.
15	18.6	Do.	43	−5.0	All roots injured; top uninjured.
18	41.2	Do.			
21	56.0	Do.	45	[1]	Tops almost dead.

[1] Dead.

Growing roots were killed at a temperature of −2° C. or lower. Growth increased slowly as the temperature was raised from 0° to about 15°; from 15° to 30° the rate of increase was very rapid. Further increase in temperature slowed down growth until 38° was reached. At that temperature growth was very slow, and at the end of the 4-day period most of the growing tips were dead. At 42° all tips were killed, while the older portion of the roots and tops were uninjured; at 45° all roots were killed and the tops showed slight injury during the period of the experiment.

These results show that roots remained alive and made some growth over a range of temperature from 0° to 38° C. and were killed at

temperatures below −2° or above 45°. Rapid growth was made at temperatures ranging from 21° to 36°, reaching a peak at 29°.

It will be noted from figure 7 that the soil temperature at 1 foot below the surface was above 21° C. during July, August, and September only, and at no time did it quite reach a temperature sufficient to promote maximum growth. It may also be noted that at no time did the soil become cold enough, by a margin of 5°, to stop growth 1 foot below the surface.

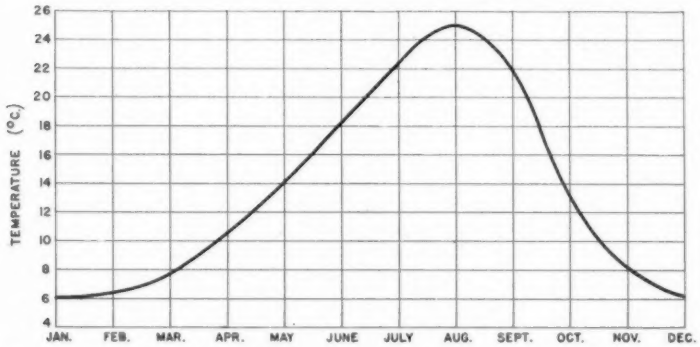

FIGURE 7.—Temperature of the soil in pecan orchard 1 foot below the surface throughout the year.

FIGURE 8.—Orientation of lateral roots of a pecan tree 10 years of age.

Roots about the base of 10-year-old and 5-year-old pecan trees were exposed by digging the soil away for a depth of 2 feet and a radius of 4 feet, as shown in figure 8. Most roots from which the soil was dug in the spring continued to grow throughout the summer. Those less than 1 mm in diameter died from drying, within a few days, and a few larger roots died when the weather became very hot, but all of those

more than about 5 mm in diameter withstood the extreme heat and dryness of summer.

The exposed roots became harder and more brittle the longer they were exposed. The cortex developed the appearance of the cortex of stems and the secondary phloem developed chlorophyll. All of the exposed roots were killed by freezing the following winter. However, none of the roots that were cold-killed died beyond the region exposed. In some cases the cutting sent up one or more shoots, thereby growing independently of the parent plant.

THE ANATOMY OF PECAN ROOTS

It is believed that a knowledge of the anatomy of the pecan roots can be applied to other tree species and that certain facts heretofore little recognized may aid in explaining problems of general application. This is especially true of the relationship of mycorrhizal fungi to the host roots. Of even greater importance is the variation in reaction of the different classes of roots to environmental conditions, especially with relation to soil moisture.

ROOTS OF THE PRIMARY PLANT BODY

All newly formed roots, regardless of size, that have not developed secondary thickening constitute the primary plant body. Such roots are always present on trees in a state of growth.

Very young pecan roots may be divided into large and small roots. each class possessing distinct anatomical characters in the primary plant body. The principal characteristics of the two types are summarized in table 3.

TABLE 3.—*Summary of the anatomical characteristics of small and large roots of the pecan*

Characteristics	Small roots	Large roots
Root cap	Only a few cells thick	Many cells thick.
Root hairs	Wanting	Wanting.
Epidermis	do	Indistinctly developed.
Cortical parenchyma	4–6 cells thick	20–40 cells thick.
Endodermis	12–16 large cells in cross section	40–100 small cells in cross section.
Pericycle	One layer of cells thick	Usually more than 1 layer of cells thick.
Primary xylem	Diarch or triarch	Pentarch to octarch.
Primary phloem	Same number of strands as in xylem of the same root.	Same number of strands as in xylem of the same root.
Pith	Absent	Present in taproots only.
Procambium	Present	Present.
Secondary thickening	May occur	Usually occurs.
Periderm	do	Do.
Length	1–10 mm	0.5–30 cm.
Diameter	0.20–0.25 mm	2–6 mm.
Mycorrhizas	Usually present	Seldom present.

Small roots are more numerous than large roots and in trees more than 1 year old are usually associated with mycorrhizal fungi. They are shorter and smaller in diameter than nonmycorrhizal roots. Large roots are comparatively thick and long, smooth, white, and usually without fungus infection (fig. 9).

A young growing nonmycorrhizal root is bluntly pointed and increases in thickness as one measures from the tip. The tips of mycorrhizal roots are usually rounded (fig. 10).

Regardless of the length of a root, all increase in the thickness of the primary plant body is attained within 2 to 8 mm of the tip, depending upon the size of the root. The extent in length of the region of enlargement is about twice the diameter of the individual root. There is an overlapping of the regions of enlargement and elongation, the latter being limited to a region of from 1 to 6 mm from the tip (fig. 9). The extent of both regions varies with the diameter of the root. In mycorrhizal roots both processes are confined to a region of less than 2 mm.

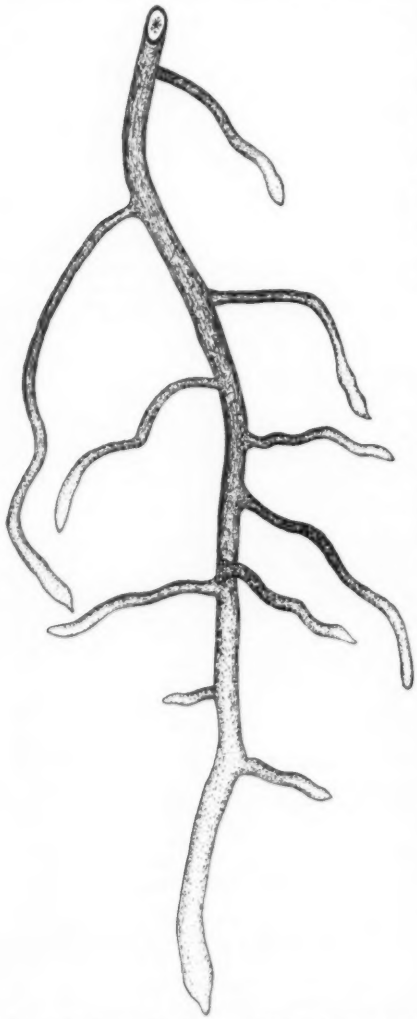

FIGURE 9.—Group of pecan roots showing absence of root hairs; the growing points are white and larger than the older portions.

ROOT CAP

A root cap is present on most growing pecan roots, but certain ephemeral nonmycorrhizal roots of the third and fourth orders have very poorly developed or no root caps. In longitudinal section the root cap appears as a parabola and consists of nonnucleate cells with firm walls (fig. 11). The cells of the root caps are renewed from a centrally located group of meristematic cells.

Mycorrhizal roots, when rather large, have a cap similar to that of nonmycorrhizal roots, but usually they are bluntly rounded and only 2 or 3 cells thick. The cap is covered with the fungous mantle, which protects the outer cells from wearing away in the course of growth in the soil.

ROOT HAIRS

Root hairs are not present on roots of pecans (figs. 9 and 11). While the writers observed an occasional epidermal cell "bulging" above the surrounding cells, several thousand examinations of roots growing under widely different humidity and temperature conditions have not revealed a single root with typical root hairs.

Epidermis

The region just back of the cap of the growing root is bound by relatively small, cylindrical parenchymal cells. The several layers directly beneath are very similar, though the cells are slightly larger. Thus, if an epidermis is present, it does not differ markedly from the cells immediately beneath. This outer layer of smaller cells is always absent from mycorrhizal and other small roots (fig. 12, *A* and *B*).

The outermost 2 or 3 layers of cells tend to act as a unit and do not develop intercellular spaces. After the cortex has collapsed and

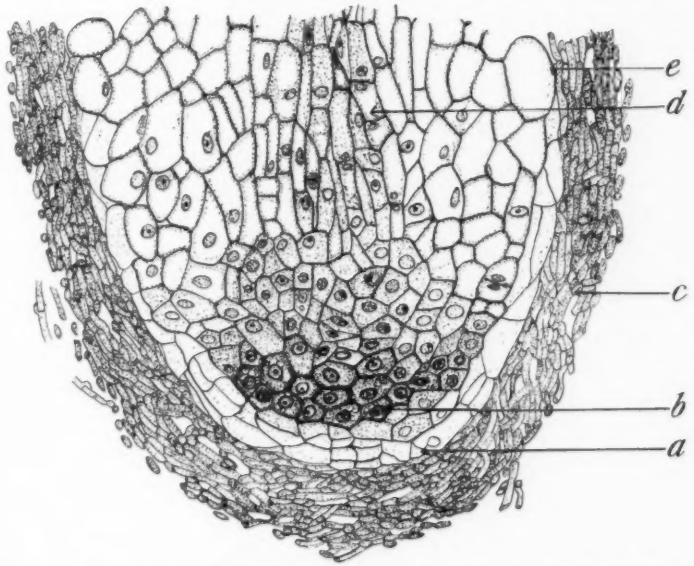

Figure 10.—Tip of a rapidly growing mycorrhizal root: *A*, Root cap; *B*, region of rapid cell division; *C*, fungous mantle; *D*, early cell elongation in region of central cylinder; *E*, early formation of cortical paren chyma. ×740.

there are no structural remains of the cortical parenchyma this region covers the root as a black wrinkled tissue.

Cortical Parenchyma

The cortical parenchyma is made up of thin-walled, parenchymal cells which are irregularly rounded with intercellular spaces between. The cells near the center of the region are the largest, and those on either side progressively smaller. The layer varies in thickness from 4 to 6 cells in mycorrhizal roots to from 20 to 40 cells in large roots, as is shown in table 3. The thickness of the cortex is the most variable character in pecan roots and is influenced to some extent by soil moisture, temperature, and the amount and nature of the nutrients. The life of the region is about 2 weeks (fig. 13).

The breaking down of the cortex is accomplished by dissolution of the walls, first, of cells near the endodermis, followed by the disintegration of cell walls and cell contents throughout the entire region. On taproots the entire primary bark usually splits longitudinally, exposing the secondary phloem in streaks. Such splitting has been observed only in roots with a pith. As a result of the breaking down of the cortex young roots are thicker at points near their tips than at points farther away.

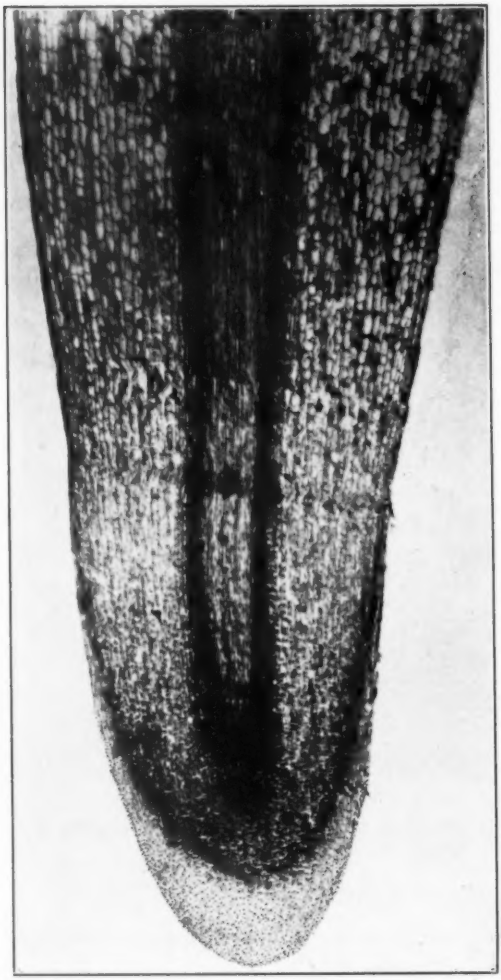

FIGURE 11.—Longitudinal section of root tip, showing the cap and other regions.

ENDODERMIS

The endodermis consists of a single layer of closely fitted, cylindrical cells which completely inclose the stele. In cross section from 14 to more than 100 cells may be observed in this region, depending upon the size of the root. On reaching full size the cells usually become filled with a gummy material, the origin and nature of which has not been fully determined (fig. 12, *C*).

PERICYCLE

The pericycle lies directly beneath the endodermis and varies in thickness in large and small roots. Mycorrhizal and other small roots have only a single layer of cells in the pericycle, while in large roots it is 2 or 3 cells in thickness.

In the formation of branch roots tangential cell division is initiated in the pericycle opposite a strand of xylem. Cell multiplication and

emergence of the new root take place in the usual manner. Apparently not all of these roots emerge at once, but some may remain latent and again become active when the tips of the parent root have been killed by adverse growing conditions. This method supplements the formation of new roots by callus formed at the base of dead root tips.

Primary Xylem and Phloem

Primary xylem and phloem develop simultaneously through specialization of primordial meristem cells in a manner typical of dicotyledonous plants. In pecans the number of xylem strands varies with the size of the root, thickness of the cortex, and root order, and is indirectly influenced to some extent by soil temperature, soil moisture, and fertility. The number of strands may vary in a

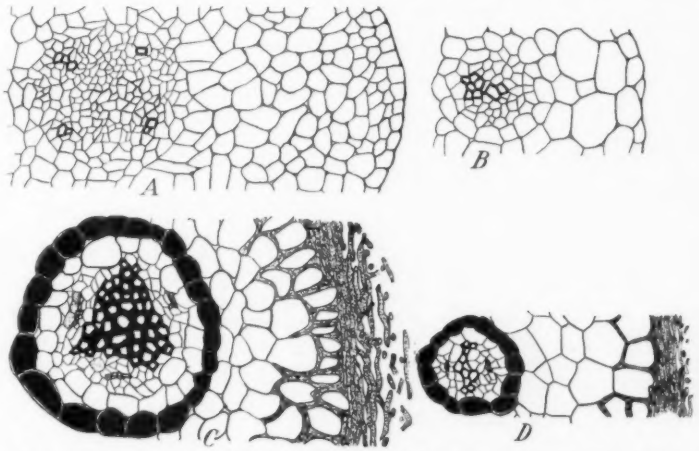

Figure 12.—Segments of cross sections of four pecan roots: *A*, Early stage in the formation of a tetrarch xylem in a large root; *B*, early stage in the formation of a diarch xylem in a small root; *C*, mycorrhizal root with a triarch xylem and well-differentiated primary phloem, pericycle, and endodermis; *D*, mycorrhyzal root with diarch xylem and well-differentiated primary phloem, pericycle, and endodermis.

single taproot from 5 to 12 by converging or dividing of the existing strands (fig. 14). The normal number of strands in a taproot appears to be 8, occupying the positions shown in figure 14, *K*. Unless growth is maintained at a rapid rate the number may drop to 7, 6, or 5. The presence of a pith seems to prevent further converging. Under some conditions one or more strands may divide and become double or treble, as shown in figure 14, *L*. In counting, the double or treble strands were considered as one.

The number of xylem strands in lateral roots is much more variable than in taproots. Pith is commonly absent, and the entire central part of these roots is primary xylem. In the absence of pith the number of strands may be reduced to as few as two. The number of strands of primary phloem corresponds to the number of strands of primary xylem, except that in cases of multiple strands of the latter, the phloem strands do not multiply correspondingly.

From tables 4 and 5 it is seen that the largest roots, with the largest stele, the greatest number of xylem strands, and the thickest cortex are associated with the presence of pith and absence of mycorrhizas. Likewise the smallest roots, with the smallest stele, the least number of xylem strands, and the thinnest cortex, are associated with the absence of pith and usually with the presence of mycorrhizal fungi. However, comparison of the anatomy of infected and uninfected small roots shows that the anatomy of small roots is not influenced by the presence of mycorrhizal fungi. Pith is present in taproots as an extension of the pith of the stem, but apparently the pericycle seldom, if ever, gives rise to a root with pith. The cells in the center of the pith region are the largest, and those surrounding are progressively smaller. They remain turgid and are usually filled with starch.

FIGURE 13.—Young pecan root with cortex partially removed, exposing the stele; the cortex sloughs off when the root is from 2 to 3 weeks old.

The cambium of pecan roots possesses a marked ability to produce callus tissue in the event of injury. This occurs when stems and roots are grafted or when root cuttings are made. Wounds made by cultivating implements also stimulate callus formation and subsequent formation of new roots or stems or both.

The cells of the secondary xylem are used for the storage of food materials, chiefly starch and water. About 60 percent of the number of cells and 90 percent of the total volume of secondary xylem are filled with starch during the greater part of the year. This region is the "wood" of roots and is more bulky, softer, and lighter in color and weight than the corresponding wood of stems. On complete drying the former lost 49.8 percent in weight as compared with 31.7 percent for the stems.

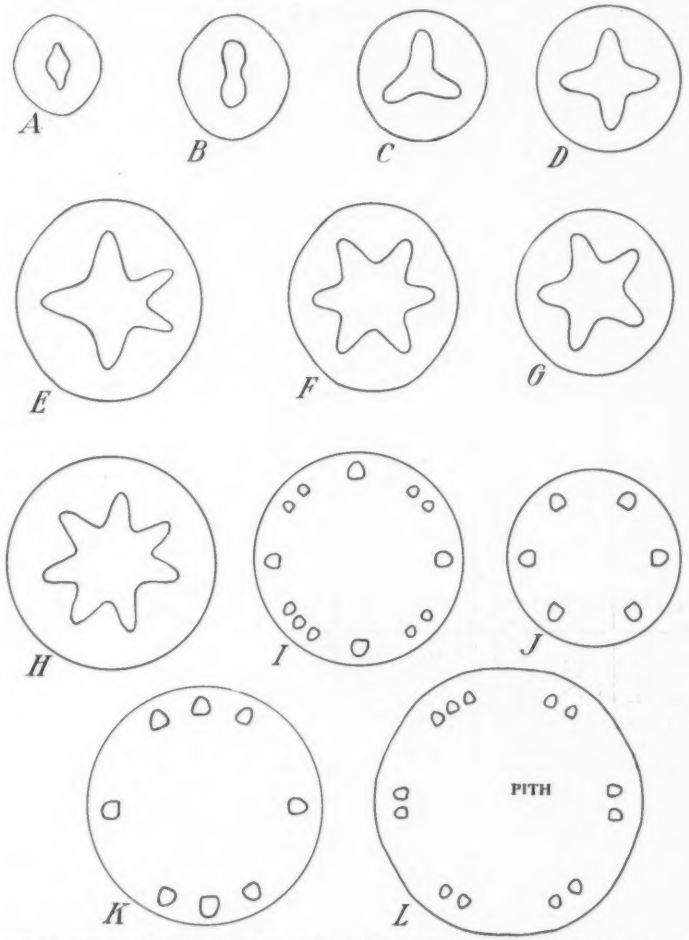

FIGURE 14.—Diagrams of cross sections of pecan roots showing 12 variations in the number and position of xylem points, and the corresponding variations in the size of roots: *A* to *H*, Roots without pith; *I* to *L*, taproots with pith; *E*, *I*, and *L*, multiple xylem strands; *K*, position of the strands in a taproot as it emerges from the shell.

TABLE 4.—*Variation in number of xylem strands in various classes of pecan roots*

Kind of roots	Number of xylem strands in roots having indicated stelar points [1]										
	Small roots			Large roots							
	2	3	4	5	6	7	8	9	10	11	12
Tap	0	0	0	0	16	8	61	13	12	2	1
Nonmycorrhizal	6	5	31	34	16	2	1	0	0	0	0
Mycorrhizal	43	26	6	0	0	0	0	0	0	0	0

[1] In studying the number of points in the stelar figure, roots were considered as belonging to 1 of the 3 classes in table 4. All taproots naturally fall in the general class of large roots, while all mycorrhizal roots naturally fall in the class of small roots. Nonmycorrhizal roots are extremely variable in size and thus fall in both classes. 8 appears to be the normal number of xylem strands in taproots, 2 of the normal number for mycorrhizal roots, and 4 and 5 for nonmycorrhizal roots.

TABLE 5.—*Relation of number of xylem points, root diameter, stele diameter, and thickness of cortex to the presence of pith and mycorrhizas*

[About 300 roots were measured]

Points in stelar figure	Diameter of root	Diameter of stele	Thickness of cortex	Pith	Mycorrhizas
	Millimeters	*Millimeters*	*Cells*		
8 [1]	3.01	1.56	21	Present	Absent.
7 [1]	3.49	1.78	29	do	Do.
6 [1]	2.43	1.08	19	do	Do.
6	2.35	.50	25	Absent	Do.
5	2.02	.45	20	do	Do.
4	1.52	.29	15	do	Do.
3	.25	.09	5	do	Present.
2	.20	.05	4	do	Do.

[1] Taproots.

As wood in roots is in the process of formation practically throughout the year, annual rings are less distinct than in stems. Heart-

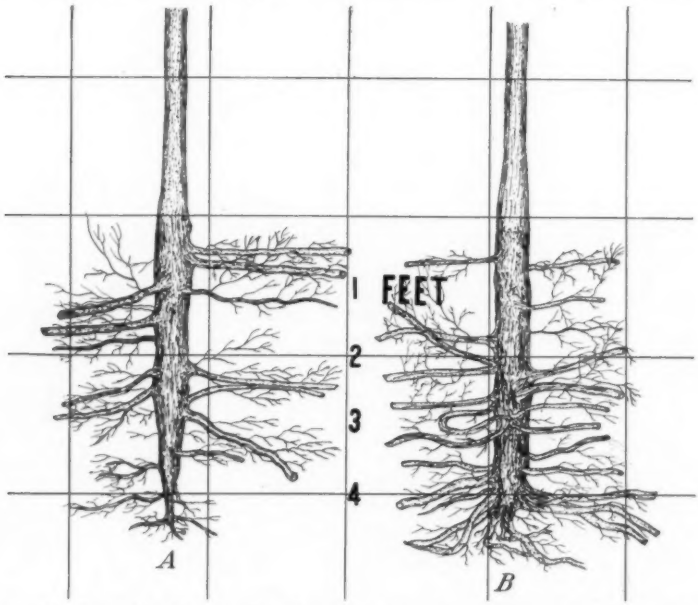

FIGURE 15.—Comparison of the orientation of lateral roots on transplanted and nontransplanted pecan trees. The nontransplanted tree *A* had 11 main laterals, while the transplanted tree *B* had 21.

wood is seldom found in pecan roots, which, when subjected to the proper conditions, decay very rapidly. The scant lignification of cell walls, the almost entire lack of tyloses, together with abundant starch and water in the xylem, afford an excellent medium for rot-producing organisms and host for insects.

Medullary cells originate from specialized cambium cells which give rise to phloem ray cells on the outside and xylem ray cells on the inside by tangential division. There are two types of ray cells in

pecan roots. The most common type of cells, as seen in cross section, is about twice as long as wide, thin-walled, and always filled with starch. Cells of the other type are about 10 times as long as wide, thin-walled, and occur in groups adjacent to the larger storage cells. The latter may or may not be used as a place of storage.

ROOT DEVELOPMENT OF TRANSPLANTED TREES

Practically all of the bearing pecan trees in the southeastern part of the United States are nursery-grown trees and were transplanted when from 3 to 6 years old. The advantages and disadvantages of this practice have not been fully studied.

The roots of several 5-year-old and 10-year-old pecan trees were dug for examination at the same time that an equal number of transplanted trees were dug. Figure 15 shows representative root systems of the two groups of trees. Differences in the depth of penetration or size of the aboveground portion were not significant, the check in growth subsequent to transplanting being completely overcome within 2 or 3 years.

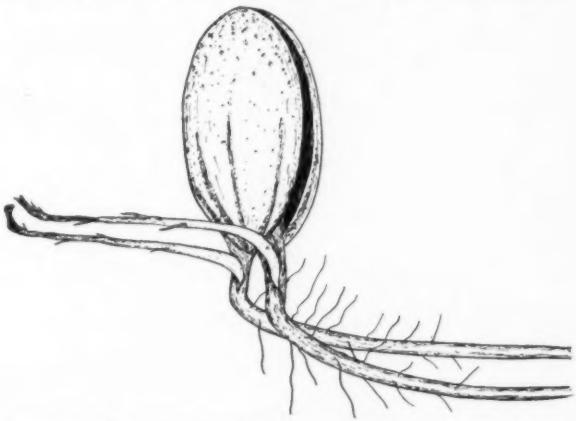

FIGURE 16.—Single pecan nut with 2 embryos giving rise to 2 taproots and 2 tops.

ABNORMALITIES IN PECAN-ROOT GROWTH

Normally a pecan germinates by splitting the shell in half at the apical end and both radicle and plumule emerging at once, but growing in opposite directions. In the course of these studies several types of abnormal behavior or roots have been observed.

MULTIPLE RADICLES

Of 10,000 germinating nuts that have been examined, about 1 out of each thousand has produced a multiple radicle (fig. 16). This character has not been correlated with any morphological character in the shell and therefore cannot be detected without either cracking or germinating the nut. Usually the 2 radicles emerged from the

shell at about the same time, but when there were 3 or more they varied widely in size and time of emerging from the shell.

MULTIPLE PLUMULES

About 1 out of 2,000 nuts when germinated has given rise to 2 plumules. More than 2 plumules have never been observed as coming from one nut. Often it was quite difficult to determine whether the nut produced 2 plumules or produced 1 plumule which later branched. All nuts which produced multiple plumules also produced multiple radicles, indicating the possibility of multiple embryos.

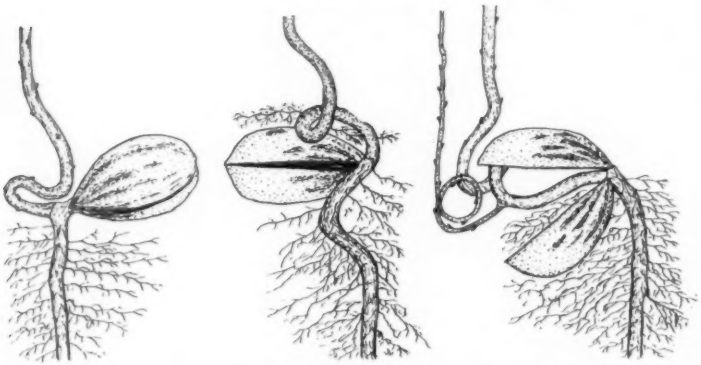

FIGURE 17.—Three germinating pecan nuts in which the plumules apparently lost their sense of direction and did not go straight upwards.

PLUMULES OR RADICLES WITH LOST SENSE OF DIRECTION

Stems normally grow upward, but numerous instances have been observed in which they did not do this. Sometimes they turned in all possible directions before finally turning upward (figs. 17 and 18). Such behavior is apparently caused by environmental conditions which have not been determined.

LATERAL ROOTS WITH LOST SENSE OF DIRECTION

A few instances have been observed where lateral or fibrous roots grew in peculiar shapes, or in positions which eventually meant their death. Figure 19 shows a root making two complete circles. In another case one root grew through another root.

ROOTS FROM COTYLEDONS

Roots are normally produced from other roots and sometimes from stems, but it is abnormal for them to be produced on cotyledons. Many times a mass of callus develops on the cotyledons near the point of attachment of the young seedling. From this callus have developed fibrous roots that function normally.

DISCUSSION

Many orchards have been thinned, and others should be thinned, because of competition among the trees. Competition begins earlier and becomes more intense with roots than with branches. The overlapping of the extremities of a few of the longest roots does not mean that thinning should follow immediately. In fact it is only when the roots meet between the rows that the soil area has become completely available to the trees. The condition existing in an orchard after the trees have been set 50 feet apart each way for about 15 years seems to approach the ideal so far as complete utilization of the soil is concerned.

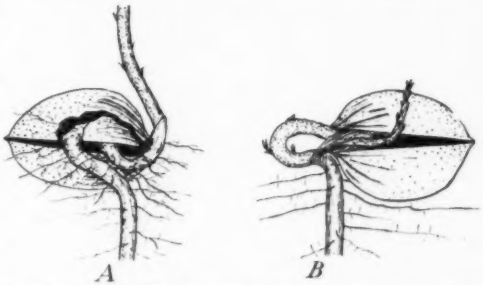

FIGURE 18.—*A*, Germinating pecan nut in which the radicle grew into the nut and then out again; *B*, germinating nut in which the plumule grew into the nut then out again.

The most economical use of fertilizers and the most advantageous types of cultivation can be carried on only when the horizontal and vertical extent of the tree roots is known. After the soil has become completely populated with roots it seems that all areas of the orchard

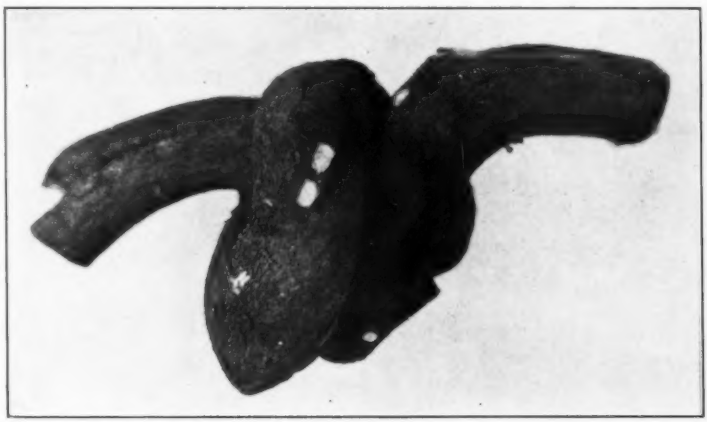

FIGURE 19.—A much curled, large pecan root which would probably have strangled itself within a few more years.

should be given equal fertilizing and cultivating treatment. The depth of cultivation will depend on the season of the year and condition of the orchard. Each cultivation disturbs feeding roots, and it is only when the good otherwise done more than compensates for the destruction of roots that the total effect of the operation is profitable. Each cultivation should have a specific object.

The time of applying fertilizers and the amount to be applied cannot be judged from a study of the roots alone. However, it is important to remember that roots grow throughout the winter, and if erosion is eliminated fertilizers can profitably be applied in the fall.

The fact that roots are almost completely inactive during droughts of several weeks duration in the summer is important. At this time of the year the nuts should be making rapid growth, and the entire leaf area of the tree must be provided with moisture when there is little to be had. All available means should be used to provide enough moisture to maintain some growth throughout the summer.

SUMMARY

The spread of pecan roots in the soil is about twice that of the branches of the same tree. Roots seldom grow more than 5 feet deep in pecan orchards in Georgia.

There is a concentration of feeding roots near the surface of the soil, where they are repeatedly killed by droughts, freezes, and injuries from cultivating implements. Fibrous roots are short in length and short-lived. They are quickly replaced when growing conditions again become favorable.

Root pruning brought about either by transplanting or by cultivation causes branching and may be advantageous.

Young pecan roots may be divided into two classes, namely, large and small roots, each possessing distinct anatomical characters. Most small roots are mycorrhizal.

Pith is present only in taproots.

Root hairs are absent from pecan roots of all classes.

A distinct epidermis is absent from pecan roots.

The number of cells composing the various regions of individual roots is exceedingly variable.

PERSISTENT STRANDS OF THE COTTON ROOT-ROT FUNGUS IN TEXAS[1]

By HOMER C. MCNAMARA, *associate agronomist*, and R. E. WESTER and K. C. GUNN, *assistant scientific aids, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The ability of the cotton root-rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) (*2*)[2] to remain in the soil in a viable and infectious condition for a period of years, even when the fields are planted to nonsusceptible crops or kept in clean fallow, has been a problem of much concern to all investigators of this disease. Further observations on the ability of this fungus to perpetuate itself in the soil for long periods were made possible during the summer of 1932, when plots that had been in clean fallow for several years or planted to nonsusceptible crops were returned to cotton. Even after a 5-year clean fallow, several centers of infection appeared in a half-acre plot during July and August.

In the planning of experimental work on the control of cotton root rot, the nature and stage of the organism that is being dealt with must be considered. Clean fallows covering from 1 to 5 years and the use of nonsusceptible crops on infested areas have furnished conditions under which it has been possible to study the fungus in the soil on both cropped and uncropped plots. The colloidal nature of the Wilson and Houston clay soils presented special opportunities for studying the fungus in its natural state of growth. The formation in these soils of cleavage surfaces into which the roots of the plants and the fungus penetrate permits a ready and satisfactory examination of both the plant roots and the fungus, an opportunity which in more friable or sandy soils would not have been possible.

The discovery of a sclerotial stage of the fungus in 1928 by King and Loomis (*3*), which affords at least a partial explanation of the longevity of the fungus in the soil, was followed the same year by another important contribution by Dana (*1*) in which he described the occurrence and infectious nature of smooth or "horsehair" strands found in primary centers[4] of infection in cotton following an 18-month clean fallow.

After an extensive search in the soil for the source of carry-over infection during 1931 and 1932 under varying soil, cropping, and tillage conditions, sclerotia were found to occur rather sparsely in plots planted continuously to cotton. Usually they were found in primary centers of infection in cotton after a cropping or tillage practice that had reduced the shallow infection, leaving only the more deep-seated or sclerotial centers to reinfest the plot when it was

[1] Received for publication Nov. 13, 1933; issued October 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 538.

[4] The term "primary centers" refers to the initial points of infection, which usually appear early in the season. From these loci of infection the disease may spread outward, or other primary centers may appear at later dates. All the plants do not die at the same time; the loss is gradual and usually represents a spread from the primary centers, which appear at points where the hold-over infection first attacks the plants.

returned to cotton. Sclerotia are often found near old tree roots and stumps when such remains are present in infested soils. These investigations indicate that sclerotia may be formed as frequently in soil continuously planted to cotton as they are in clean fallows. However, the primary centers of infection that appear when cotton follows clean fallows or nonsusceptible crops facilitate the location of sclerotia. In such plots the first plants to die afford some indication of the presence of sclerotia, since the first dead plants are limited to centers where the carry-over infection was due either to deep-seated strands or to sclerotia. In plots planted continuously to cotton the great majority of dead plants result from infection carried over by strands; therefore these dead plants do not indicate the presence of sclerotia.

From a very large number of primary centers in continuous cotton plots explored during the seasons of 1931 and 1932, only two colonies of viable sclerotia were found. Both centers were found in 1932, and the first colony was located on June 11, 1932, beneath the first plant to die on the United States Cotton Breeding Field Station, Greenville, Tex. At least 75 more primary centers of infection in continuous cotton plots were examined during the succeeding 3 weeks, but not a single sclerotium was found, nor was there evidence of any having been formed in these centers. Further explorations throughout the summer failed to reveal the presence of viable sclerotia in continuous cotton plots, although sclerotial "casts" were found on several occasions. The casts, which remain for several years after the sclerotia have disappeared, prove conclusively that sclerotia were present at some previous period. Since only two colonies of viable sclerotia were found in a 2-year search of plots planted continuously to cotton, it would seem certain that most of the infection in such plots is carried over in the soil by some means other than the sclerotial stage of the fungus.

King and Loomis (*3*) have discussed the analogy between *Phymatotrichum omnivorum* and *Armillaria mellea* (Vahl) Fr., which leads a saprophytic existence on dead stumps and roots but which under favorable conditions becomes a parasite. This fungus relies largely on rhizomorphs for its dissemination, and its cordlike rhizomorphs have been referred to as "in reality elongate sclerotia" which radiate through the soil. With the knowledge that the strands of *P. omnivorum* may persist in the soil for several years and may remain capable of producing infection, the analogy is even more striking. Viable tufted strands of *P. omnivorum* have been frequently found emerging from colonies of old sclerotial casts in which no viable sclerotia were found. Old strands that were removed from clean fallows during August and used as inoculum on cotton plants in the field produced infection, killing the plants within about 30 days.

STRANDS IN FIRST-YEAR CLEAN FALLOW

Examinations of the soil in a first-year clean fallow, beginning July 18, 1932, and continuing throughout the summer, yielded numerous dark-colored strands in situ upon old dead roots of the previous crops. Some of these strands were on the roots of cotton plants of the 1931 crop; others were unquestionably older. When removed from the soil in July and August and placed on filter paper in a moist chamber

A, Strands from a freshly wilted cotton plant. They are light in color and are surrounded with an outer network of acicular branches with right-angle cells. *B*, Old strands, removed from dead roots of previous cotton crops, taken from a first-year clean fallow on July 26. The older strands are darker in color and show fewer acicular branches on the outside. ×20.

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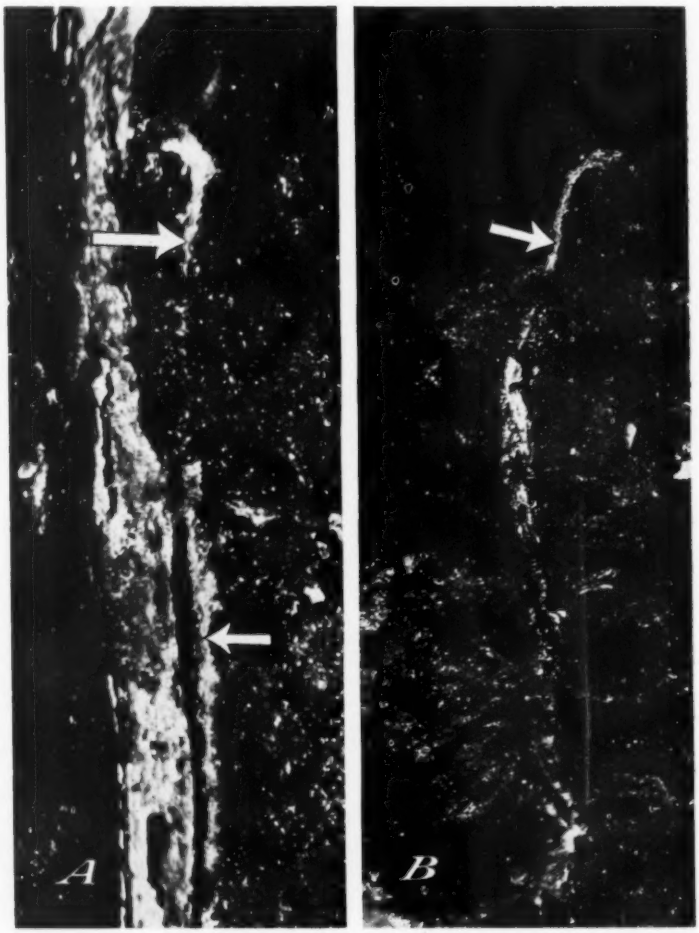
Persistent Strands of Cotton Root-Rot Fungus PLATE 2



A, Old strand of *Phymatotrichum omnivorum* (indicated by arrows) in original position, attached to decayed cotton root. *B*, Old strand projecting beyond broken end of dead cotton root. These strands were removed from the soil in a first-year clean fallow in August 1932. When placed in moist chambers strands like these put out new growth from the ends in 12 to 48 hours; when exposed and reburied in the soil they germinated within 1 to 3 days. ×20.

the strands threw out overnight a vigorous growth from the broken ends. These strands were darker in color than fresh strands and were rather sparsely clad with an outer growth of acicular branches, which are common to fresh strands (pl. 1). In the Wilson clay soil the old strands are frequently coated over with a gelatinous mass of colloidal material, which makes them very difficult to identify with the unaided eye. The strands are more flexible than the dead roots to which they are usually attached and may be disclosed by breaking apart the decayed roots, thus leaving them exposed at the break, or showing them firmly embedded in the soil surrounding the root (pl. 2).

Continued examinations in the first-year fallow plot failed to reveal viable sclerotia, although one colony of sclerotial casts was found on August 14. When this plot was in cotton the previous season and the number of dead plants was very large, no traces of sclerotia were found. On August 12 fresh tufts of mycelium were found about 7 inches below the surface on a dead taproot, indicating that the fungus not only remains alive on the old roots but may also make some growth on such material. This root had been cut off about 5 inches below the surface when the land was bedded during the previous winter. The growth was interlacing an earthworm boring, which may have served to provide aeration. In this first-year clean fallow only two cases were found in which the fungus was making active growth on the dead root material in the soil, but such growth on the dead cotton roots and stalks in infested cotton plots was very profuse during the second week of August.

Neal and Wester (*7*) have shown that the fungus does not grow under anaerobic conditions in the laboratory. Assuming that this is true under natural conditions, the organism may remain in the soil in a viable condition until such time as it can be supplied with oxygen or other necessary stimulus for renewing growth. Old strands dug up from a first-year fallow plot on August 7 and then reburied showed new growth from the ends 1 to 3 days later. Strands are undoubtedly much more resistant to decay or less parasitized than plant roots, as shown repeatedly by old strands occupying empty root channels or remaining embedded in the soil along the root channels after the roots are completely decayed. The outer part of the older smooth strands is made up of a close-fitting compact mass of cells. The network of hyphae on the outside of the strands disappears, leaving the core still viable and infectious. Plate 1 shows fresh strands from a plant dying of root rot and also strands that are about 1 year old. The latter were taken July 26 from a plot that was in clean fallow for the first year, following cotton in 1931.

Neal and McLean (*6*) reported germination of strands taken from beneath a spore mat, and also subterranean strands collected as late as December 10, 1929. These writers advance the opinion that the smooth strands found by Dana (*1*) at Temple, Tex., and those found by Ratliffe (*8*) at San Antonio, Tex., in July, "may have originated from sclerotia formed in the soil the preceding season." Neal and McLean (*6*) also state that—

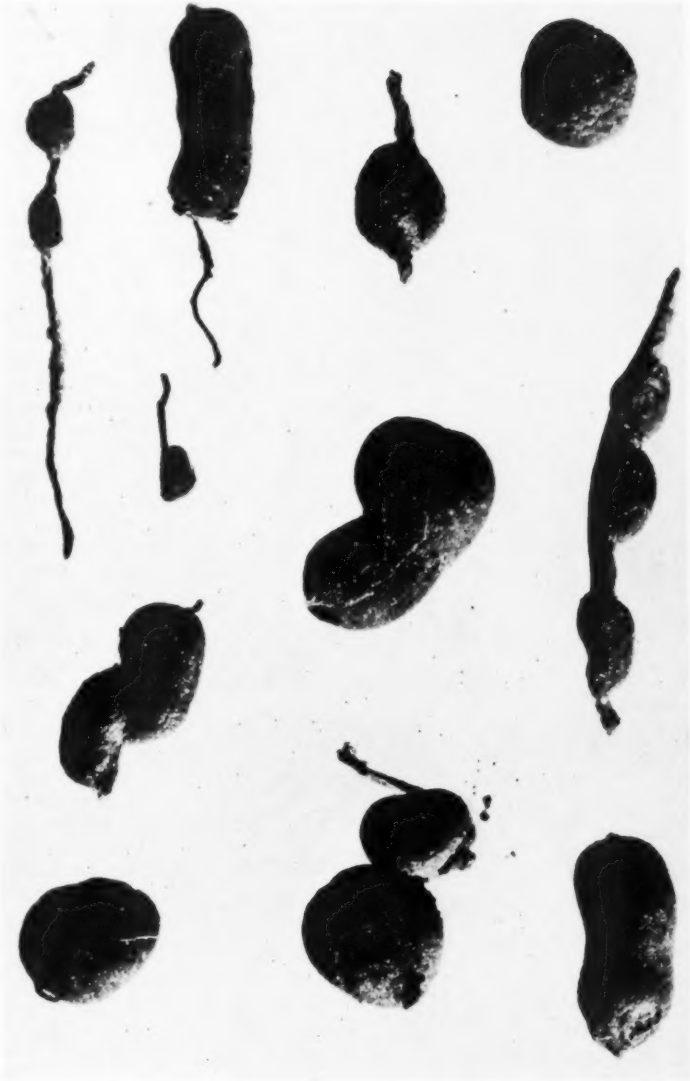
> the strands of the fungus found on infested cotton roots and other plants do not maintain their viability for long periods under field conditions, as repeated efforts to use them as inoculum in both greenhouse and field experiments or for isolating the fungus have met with failure.

King and Loomis (*3*) state that they have never been able to obtain growth from "detached fragments of strands from soil", nor from "those entirely separated from the surface of infected roots." Taubenhaus and Ezekiel (*9*) are of the opinion that the root-rot organism overwinters on live cotton roots. They state that *Phymatotrichum omnivorum* was "viable on living although infected roots, but not on roots that were infected and decayed."

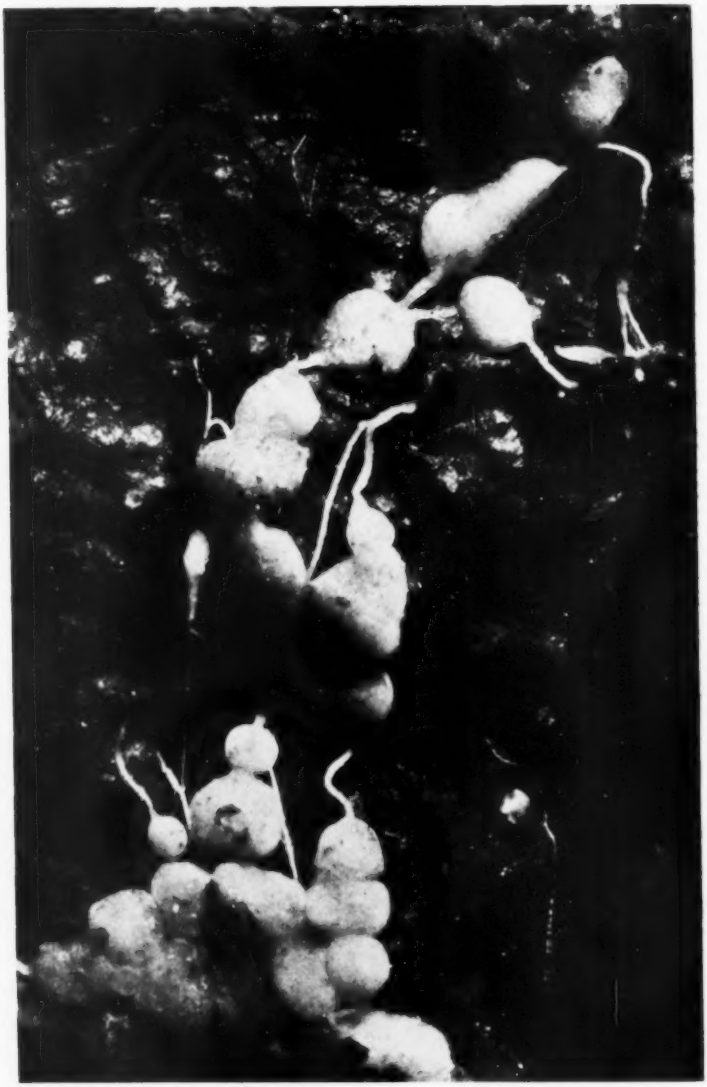
It is not possible to determine with accuracy just what changes in the growth of the fungus may take place in the soil from season to season. However, hundreds of excavations in primary centers of infection in plots planted continuously to cotton and in plots of first-year clean fallows, as well as in 3-year and 5-year clean fallows followed by cotton, show almost without exception that the strands were attached to old cotton roots or were occupying old root channels in what appeared to be their original positions (pl. 2). The scarcity of sclerotia and sclerotial casts in the plots planted continuously to cotton and in the first-year fallow is further evidence for the belief that these strands remain in their original positions and in a viable condition until some favorable stimulus is encountered, causing them to put forth new growth. Strands taken from a first-year clean-fallow plot during July and August 1932 put out new growth that was visible microscopically in 4 to 12 hours. At least during these 2 months no difficulty was encountered in germinating old strands removed from the soil—strands that in all probability were from 1 to 3 years old. There is a possibility that the strands have some seasonal growth response that might account for the failure of some investigators to germinate them, although sclerotia germinate in the laboratory under the full range of seasonal conditions (*3*).

STRANDS IN PLOTS PLANTED TO COTTON AFTER 2 YEARS IN OATS

Soil examinations were first made on July 22 in an infested area that had been planted to oats during 1930 and 1931 and returned to cotton in 1932. This area had received two 12-inch subsoilings each summer following the oat crop, in addition to the regular breaking of the stubble. While the first primary centers that appeared were being excavated to determine the source of carry-over infection, viable strands were found with the decayed roots of the cotton plants that had grown on this plot in 1929 or previously. Sclerotia were found in only one of the centers examined between July 22 and August 30, whereas in a number of the centers the only form of the fungus found was strands that apparently had been attached to the dead roots of the plants killed, probably in 1929. It seems clear that a majority of the centers of infection in this field were due to strand carry-over rather than to sclerotia. Since this plot had been kept entirely free from weeds during the summers of 1930 and 1931 and since the strands were found with the dead cotton roots, there is every reason to presume that they had remained intact in the soil for a period of at least 3 years, and possibly longer.



SCLEROTIA FORMED IN NATURE AND REMOVED ON JULY 27 FROM PRIMARY CENTERS OF INFECTION IN COTTON FOLLOWING A 5-YEAR CLEAN FALLOW (1927-31, INCLUSIVE). ×20.



FRESHLY FORMED SCLEROTIA IN THEIR NATURAL POSITION BETWEEN THE CLEAVAGE PLANES OF WILSON CLAY SOIL.

These sclerotia were found in a primary center of infection in cotton following a 5-year clean fallow, on July 30, 1932. After removal from the soil they turned light brown within a few days. ×20.

STRANDS IN PLOTS PLANTED TO COTTON AFTER A 5-YEAR CLEAN FALLOW

Further observations upon strand viability were made during the summer of 1932 in a search for the seat of root-rot infection in cotton following a 5-year clean fallow on a plot of the station at Greenville, Tex. The plot was kept in clean fallow for 5 successive years, 1927 to 1931 inclusive, and no weeds or other vegetation were allowed to grow on it. In 1932 it was returned to cotton. On July 9 three plants in different centers were dead and later several other infection centers appeared. The occurrence of sclerotia in these centers was not unexpected and corroborates the results of previous investigations by McNamara and Hooton (*5*). The earth was removed from these three centers during the week of July 9 to 16, and sclerotia and casts were found in abundance in each (pl. 3). The sclerotia were well preserved and a few showed a little new growth. Very dark-colored old strands were found interlacing each colony of sclerotia, and in many cases lying between the cleavage planes of the soil and along old root channels. In several cases the broken ends of the strands protruding from cracks were found supporting a tufted growth of active mycelium. This growth condition was observed in the strands much more commonly than among the sclerotia. Many strands and sclerotia were taken from the soil in areas not penetrated by the roots of the 1932 crop. Evidently some of the sclerotia and strands were several years old. The strands and sclerotia of each colony were probably of the same age, and both may have been regenerated from older colonies of sclerotia. Some of the strands, however, that were occupying old root channels may have been formed on cotton roots the last year that cotton was grown on the plot. A pure culture was obtained from an old strand taken from a primary center that developed in a plot of cotton following 5 years of clean fallow. Cotton plants in the field that were inoculated with this culture during August died of root rot in 30 to 50 days.

The old strands in nature are a reddish brown to dark brown, resembling to the unaided eye, and sometimes under a hand lens, the remains of small rootlets. However, with higher magnification their true identity can be readily determined. Their close resemblance to dead rootlets and the sparse growth of acicular cells on the outer part of the strand, together with their minute size, make them difficult to find in some soils. These old strands represent the large central core of the original "fuzzy" strand, from which the outer covering of acicular branches has largely decayed or otherwise become detached, leaving smoother strands which are sclerotial in their outer cellular structure and function as sclerotia insofar as reproducing infection is concerned, although new growth originates from the elongated septate cells of the central part of the strand.

In contrast to the more deeply seated infections of the far Southwest, the strands at Greenville, Tex., were found most abundant in the surface foot of soil, a position that makes them more accessible to tillage operations and to soil disinfectants.

FORMATION OF NEW SCLEROTIA

During the period from July 30 to August 13, freshly formed sclerotia were found in abundance in several different primary centers of infection in cotton following 3-year and 5-year clean fallows. These sclerotia were formed about 6 inches below the surface and in every case were found above colonies of older sclerotia or casts 8 to 14 inches below the surface. King, Loomis, and Hope (*4*) describe the regeneration of sclerotia in culture by a budding process in which new sclerotia are formed, and also report occasional old strands developing swellings and producing a few sclerotia in the same manner as active strands. While the young sclerotia that were found were not directly the result of budding, they apparently were the product of new growth originating from another colony. Freshly formed sclerotia are pearly white in color; the natural position they occupy between the cleavage planes of the soil is shown in plate 4. New sclerotia were also forming in old root channels alongside the remains of the old roots. When exposed to the air, the new sclerotia and strands, even though very small, soon put out new growth.

A portion of one of these newly formed colonies of sclerotia was left in the soil and covered with loose moist earth. On August 16 the remainder of the colony freshly formed on July 30 was removed. In the meantime the sclerotia had changed from a pearly white to a light brown and where exposed to aeration had thrown out a dense growth of acicular branches similar to the growth on the outer part of strands (pl. 5).

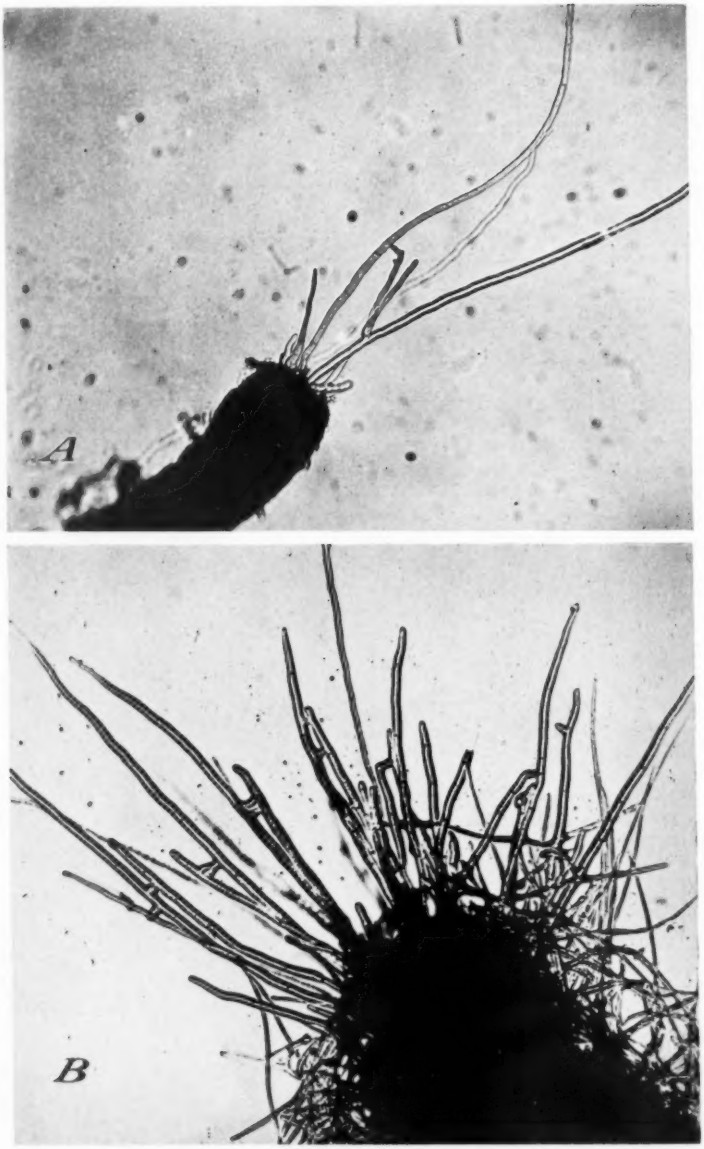
GROWTH OF HYPHAE FROM STRANDS

In testing the old strands for viability, an interesting growth habit was observed in practically every strand that germinated. On July 18, old strands taken from a plot that had been in clean fallow for 5 years were cut into very small pieces and placed in a hanging drop on a well slide, where subsequent growth development could be observed from day to day. Some of the strands failed to put out new growth at once, but many others showed germination within 4 to 12 hours after being removed from the soil. In most cases the new growth came from the ends of the strands.

The new growth from the broken ends of the strands consisted of parallel or radiating branches with elongated cells which anastomosed freely. In some cases the unions took place between the first cells to appear and in others after a number of new cells had been formed, as is shown in plate 6. The union of two or more branches through the side wall of the cells was not only common in the new growth from the broken ends of the old strands, but appeared as a regular habit in all the strands germinated during the latter part of July. United branches occurred also in the new growth arising from the sides of the strands but not with the regularity of those in the growth from the ends. The cell content of the branches developing from the ends of the strands appeared to be rather granular in contrast to that of the hyaline acicular branches. The new growth was very rapid and numerous cases of cell fusion took place within 12 to 48 hours. A break in the side or the exposure of fresh tissue at the ends of the strands apparently induces growth if the strand is viable. As the

SCLEROTIA FROM A PART OF THE SAME COLONY AS THAT SHOWN IN PLATE 4 BUT LEFT IN THE SOIL FOR AN ADDITIONAL 17-DAY PERIOD AND REMOVED ON AUGUST 16.

During the 17-day period these sclerotia put out a dense fuzzy new growth and changed to a darker color. ×20.



CHARACTERISTIC NEW GROWTH FROM ENDS OF STRANDS.

A, Typical early growth from the end of an old strand. ×135. Growth usually developed from the ends of the strands and was generally characterized by the common occurrence of united hyphae with granular filaments. *B*, Numerous cases of anastomosing hyphae in new growth from the end of a strand. ×405.

new growth continued, the anastomosing of neighboring cells became less frequent and finally the long acicular type of branches was produced.

SUMMARY

The ability of the cotton root-rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) to remain in the soil in a viable and infectious condition for a period of years, even when the fields are planted to nonsusceptible crops or kept in clean fallow, has been a problem of much concern to investigators of this disease.

During 1931 and 1932 opportunity was presented on the United States Cotton Breeding Field Station at Greenville, Tex., to study further the habits of the fungus in nature. Extensive searches in the soil for the source of carry-over infection were made in a number of infested plots that had been handled under different cropping and tillage conditions and that therefore permitted at least a minimum estimate as to the age and character of the carry-over infection.

The colloidal nature of the Wilson and Houston clay soils, in which the examinations were made, presented an unusual opportunity to study both the fungus and the plant-root development in their natural state of growth.

An examination in 1931 and 1932 of a very large number of primary centers of infection in plots planted continuously to cotton revealed the fact that the carry-over infection was not due entirely to sclerotia and that much of the infection overwintered in the soil as strands rather than as sclerotia.

Examination of a plot that was in clean fallow for the first year yielded numerous viable strands throughout the summer and fall of 1932. These strands were located, as a rule, along the old dead roots of the plants that had been destroyed by root rot a year or more before. In the clean fallows only two cases were found in which the fungus showed signs of growth on dead roots, whereas such growth was profuse in infested cotton plots.

On a plot which had been in oats in 1930 and 1931 and which had been kept in clean fallow after the crops were harvested, viable old strands were found throughout the summer of 1932 in and near primary centers of infection. These strands were found in situ upon the decayed remains of the old cotton roots that probably had been destroyed by the fungus 3 years or more before. In some cases they were occupying the empty root channels left by former plantings. When these strands were removed from the soil and placed in moist chambers or when they were exposed and reburied in the soil, they readily put out new growth.

Old strands found interlacing a colony of sclerotia in a plot that had been in clean fallow for 5 years were also viable. The age of the strands associated with colonies of sclerotia could not be determined accurately, as they may have been the product of regenerated growth, from the original colony, subsequent to the fallow treatment.

In contrast to the more deeply seated infections of the southwestern United States, the strands at Greenville, Tex., were found most abundant in the surface foot of soil, a position which makes them more accessible to tillage control or to soil disinfectants.

From July 30 to August 13 freshly formed sclerotia were found in abundance in primary centers of infection following 3-year and 5-year

clean fallows. Older sclerotia and sclerotial casts were found within a few inches of every group of new sclerotia.

The new growth, which usually took place from the ends of the strands, was characterized by radiating or parallel growth of elongated cells that freely anastomosed during the early growth stages. As this growth proceeded, the union of cells became less frequent and the more typical acicular branches with right-angled cells appeared.

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MORPHOLOGY AND LIFE HISTORY OF THE COTTON ROOT-ROT FUNGUS IN TEXAS[1]

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INTRODUCTION

Various investigators (*3, 6, 14, 17, 18, 21*)[2] have contributed information in recent years on the morphology and life history of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug. The first complete description of types of hyphae observed in cultures and on the roots of susceptible hosts was published by Duggar (*3*) in 1916. Following these reports, the discovery by King and Loomis (*6*) of a sclerotial stage of the fungus in laboratory cultures and later the finding of viable sclerotia in the soil of infested cotton fields in Texas (*9*), and in Arizona and California (*4, 5*) have been the impetus for additional investigations regarding the morphology and viability of different stages of the fungus and their specific roles in the perpetuation of the disease in the soil.

Such studies were made by the writers (*13*) from 1929 to 1932, and the additional knowledge obtained concerning the life history of the organism has served to explain more completely the manner in which the disease persists in the soil, especially in the infested districts of Texas, the region of widest prevalence and greatest damage.

Three stages of development are known to occur in the life history of the fungus. These are: (1) The vegetative or ozonium stage, which spreads through the soil and attacks the roots during the growing season; (2) the sclerotial or resting stage, one of the principal means of persisting and overwintering in the soil; and (3) the conidial stage, which frequently occurs above ground under special conditions of shade and moisture, but the function of which is yet unknown. Attempts to germinate conidia have been unsuccessful, and there is as yet no evidence that they cause infection.

MORPHOLOGICAL STUDIES OF THE OZONIUM STAGE

On the surface of infected cotton roots and various culture media three diverse types of hyphae are readily observed. There are the large-celled, acicular-branched, and hyphal strands, as well as certain intermediate types, the latter being frequently encountered in cultures.

LARGE-CELLED HYPHAE

The large-celled hyphae are the initial stage of growth of the fungus and are the type involved in the penetration of the host tissue (pl. 1, *A*). The type not only includes a greater part of the white mycelium present on the surface of infected roots, but is likewise abundant on the margins of conidial areas where, according to Duggar (*3*), individ-

[1] Received for publication Jan. 19, 1934; issued October 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 547.

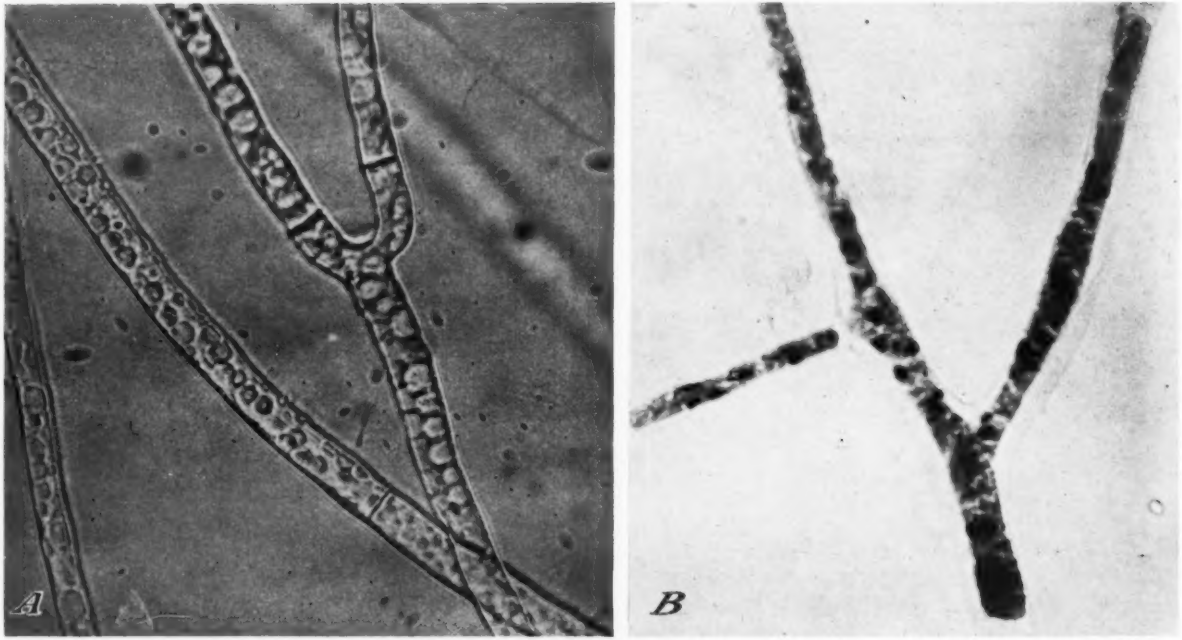
ual cells measure 20μ in diameter with cross walls 60μ to 120μ apart. In measurements made by the writers, single cells of the large-celled hyphae obtained from infected cotton roots after 24 hours' incubation in moist chambers averaged 8μ in diameter, with cross walls 72μ apart. The cells resemble those of *Rhizoctonia*, but the branching of filaments is frequently dichotomous as well as right-angled (pl. 2, *A*). The protoplasmic contents of individual cells, as revealed in unstained material from fresh cultures, are multinucleate and appear as hyaline, granular, oval bodies which refract light readily, giving the cells a vacuolate appearance (pl. 1, *B*). In material stained in iron-alum haematoxylin, the nuclei are more clearly defined and range in number from 4 to 20 in a single hypha (pl. 2, *B*). As indicated in previous reports (*10*, *12*, *19*) a common form of growth of the large-celled hyphae is the fusion and anastomosing of individual cells, which seem to represent early stages in the development of the strands of the fungus preceding the development of sclerotia. More recently the same character of growth has been observed in germinating strands and sclerotia, the connecting projections of individual hyphae resembling in many respects the conjugations of certain filamentous algae. In studying these growth phenomena, however, no actual nuclear fusion has been noted, and there appears to be no indication of a reproductive function.

FIGURE 1.—Well-differentiated coarse brown strands of *Phymatotrichum omnivorum* covering section of decayed cotton root. ×13.

HYPHAL STRANDS

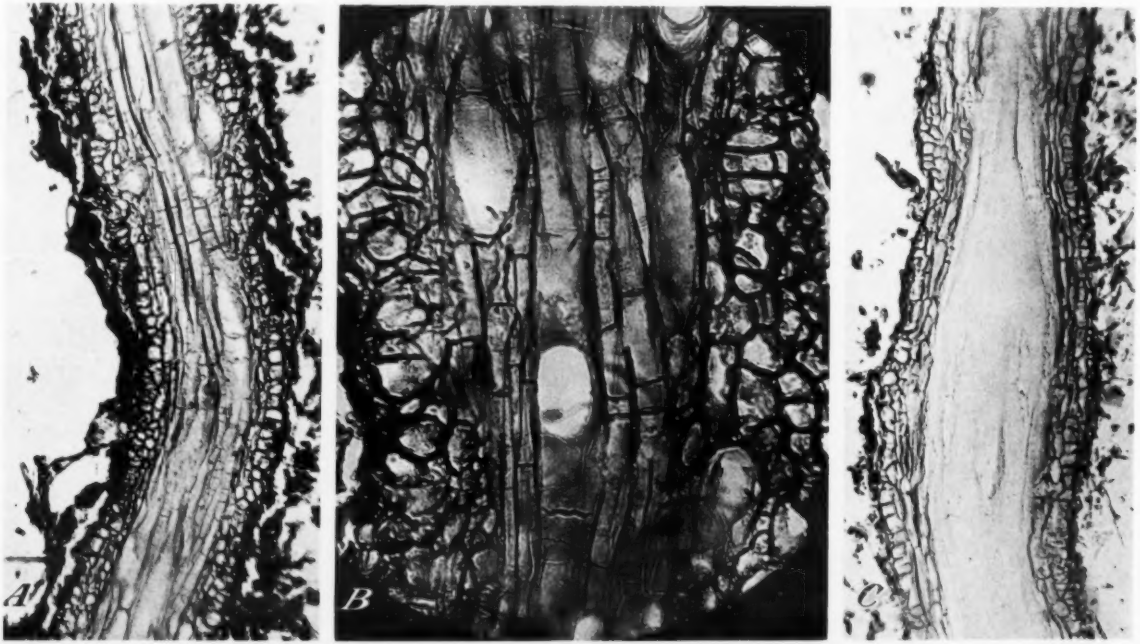
As growth proceeds, the large-celled mycelium, through fusion and anastomosing of individual cells, as previously

A, Cross section of root of cotton seedling showing intercellular and intracellular invasion of the parenchyma by the large-celled hyphae of *Phymatotrichum omnivorum* 11 days after inoculation. ×640. *B*, Large-celled hyphae of *Phymatotrichum omnivorum* showing the fusion and anastomosing and the vacuolate appearance of the protoplasmic contents of individual cells. ×640.



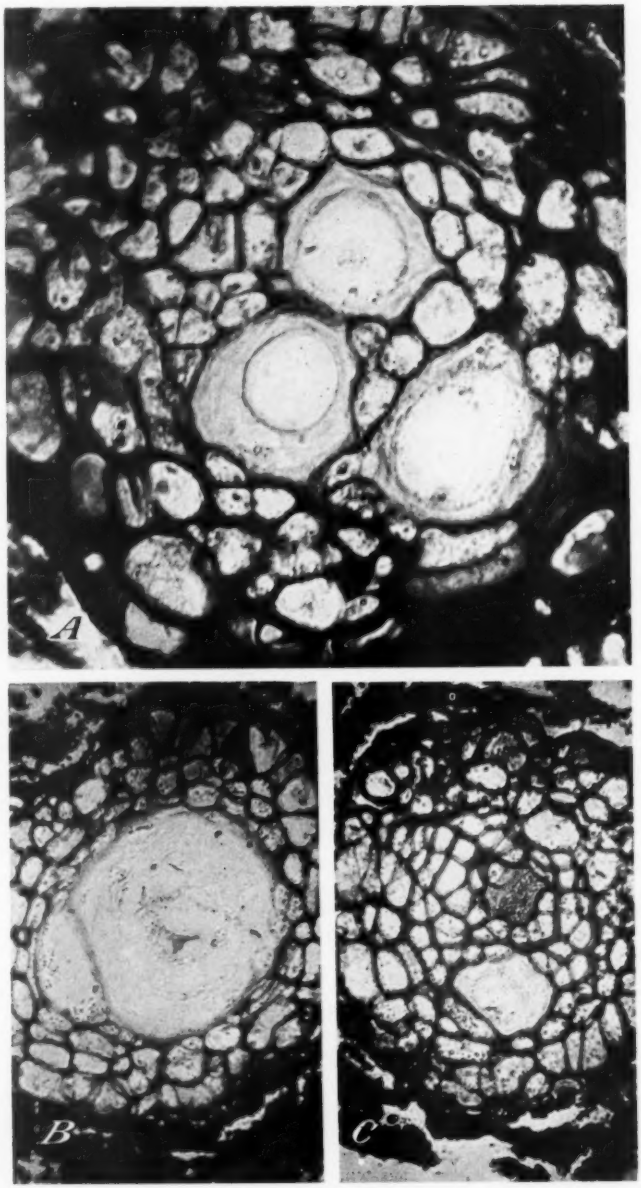
BRANCHING OF FILAMENTS OF PHYMATOTRICHUM OMNIVORUM.

A, Large-celled hyphae (unstained) showing dichotomous branching. ×640. *B*, Large-celled hyphae (stained in iron-alum haematoxylin) showing right-angle branching and multinucleate appearance of the cells. ×640.



LONGITUDINAL SECTIONS OF STRANDS OF PHYMATOTRICHUM OMNIVORUM RECOVERED IN JULY FROM AN INFESTED AREA IN A 1-YEAR FALLOW PLOT.

A, Section showing the large central septate hypha, surrounded by smaller thin-walled parallel cells, and the outer thick-walled cells comprising the cortical layer. ×165. *B*, Highly magnified portion of *A*. ×325. *C*, Section showing continuous or hollow appearance of the central hypha of the strand. ×165.



CROSS SECTIONS OF THE PHYMATOTRICHUM OMNIVORUM STRANDS SHOWN IN PLATE 3.

A, Strand section showing three large central hyphae and the surrounding thin-walled cells bordered by the thick-walled cells of the cortical layer. ×640. *B*, Strand section showing division of the central hypha. ×335. *C*, Strand section showing an entirely cellular structure similar to sclerotia. ×325.

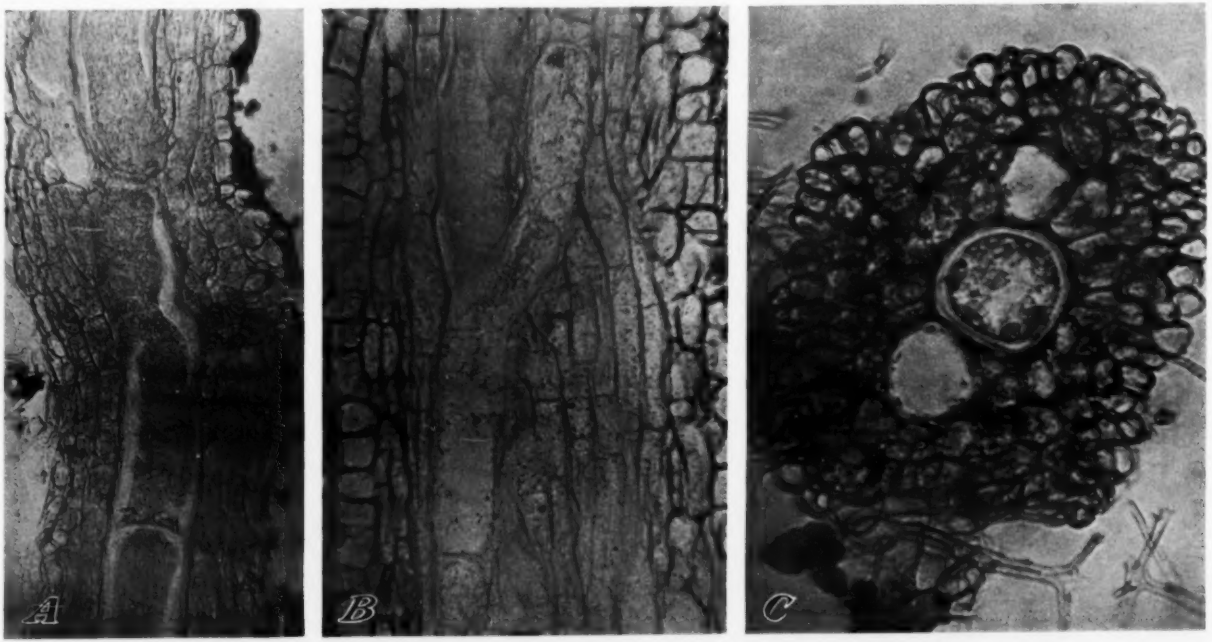


PLATE 5

LONGITUDINAL AND CROSS SECTIONS OF STRANDS OF PHYMATOTRICHUM OMNIVORUM OBTAINED BENEATH CONIDIAL MATS.

A, Longitudinal section showing the large highly granular central hypha and surrounding cell layers. ×640. *B*, Longitudinal section showing branching of the central hypha. ×640. *C*, Cross section showing the large central hypha, and two smaller but similar hyphae formed as a result of branching and cell division of the original central portion. Note the well-differentiated irregular-shaped thin-walled cells surrounding the large hypha and the compact thick-walled cells of the cortical layer. The attached acicular branches are also present at the margins. ×640.

A, Young *Phymatotrichum omnivorum* sclerotia forming through elongate swelling of the strands in soil culture 12 days after inoculation. Note chainlike and clustered disposition on the strands. ×2. *B*, Young sclerotia (8 days old) from which the section shown in plate 7, *A*, was prepared. ×15.

mentioned, ultimately differentiates into coarse brown strands (fig. 1). In agar cultures or infected roots kept in a moist chamber, fully developed strands occur within 2 or 3 days. They are white when first formed, but as they mature they become ochraceous buff to cinnamon brown.

Inasmuch as the hyphal strands are the structures necessary for the formation of the sclerotia, the latter being formed as enlargements along the strands either singly, in chains, or in clusters, the writers have made a detailed study of their morphology. Moreover, in view of previous reports (*3*, *15*, *16*, *19*) of their importance in the persistence of the disease in the soil, it appeared desirable to compare their cellular structure with that of sclerotia and to record the morphological changes at different stages of development. For this study, strands were collected from (1) decayed cotton roots in fallow plots, (2) soil cultures, and (3) the soil underlying conidial mats, and permanent slides were prepared by the paraffin method. A variety of fixatives were used, the most satisfactory results being secured with a modification of Navashin's solution, discussed in a recent cytological paper by Beal (*1*). Sections, ranging in thickness from 10μ to 20μ, were stained for the most part in iron-alum haematoxylin, Flemming's triple stain, crystal violet, or Delafield's haematoxylin.

The cellular structure of strands that were obtained during July 1932 from the soil of infested cotton plots kept fallow for 1 year is shown in longitudinal and cross section in plates 3 and 4. These strands were composed of one large central hypha surrounded by 2 or 3 layers of elongated, thin-walled, parallel hyphae which did not stain readily. Bordering the hyphae were 3 or 4 layers of rather compact thick-walled cells similar to the epidermal layer of sclerotia. The cells of the central hypha as a rule were definitely septate and variable, especially in the distance between cross walls, though the central portion of some strands was continuous or hollow, showing no differentiation of cellular structure. Other strands were entirely cellular, revealing in general the morphology of mature sclerotia, the central cells being typically thin-walled and parenchymalike.

In young strands prepared from cultures 8 days old and in those underlying mature conidial mats, a somewhat similar cellular structure was observed. However, as a general rule, the central portion consists of a single large hypha (pl. 5) described by Peltier, King, and Samson (*14*) as highly granular in content, with septa in most cases averaging about 325μ apart. As growth of the strand proceeds, the central hypha branches, and examinations of cross sections at this stage of development usually show the original large hypha and two similar but smaller hyphae. Moreover, it is apparent from these studies that the 2 or 3 bands of surrounding, smaller, thin-walled cells, as well as the cortical layer, result from the growth, branching, and rapid division of the cells of this central portion of the strand. In these strands, the cortical layer of irregular-shaped, thick-walled cells is frequently reduced and in some cases may even be entirely lacking. It was also observed that the age of strands apparently cannot be determined by the presence or absence of the right-angled acicular-branched hyphae, which are a regular feature of strands occurring on the roots of freshly wilted plants and on those under-

lying conidial mats. Strands obtained from the soil, even in areas where nonsusceptible crops have been planted for prolonged periods, may be surrounded either by the radiating acicular-branched hyphae or may be "bald" or smooth, as Dana (*2*) observed.

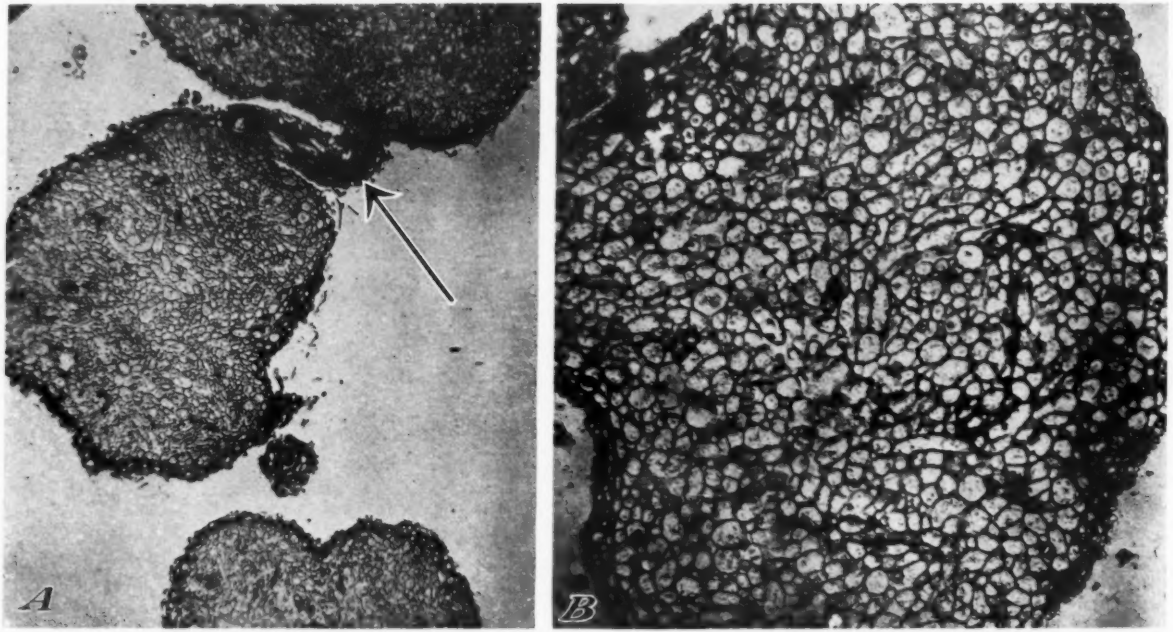
Peltier, King, and Samson (*14*) have compared the strands of the root-rot fungus to the cordlike rhizomorphs of some of the Basidiomycetes. Undoubtedly these strands show a similar morphology and physiological function. Although not comparable in size to the large rhizomorphs of *Armillaria mellea* (Vahl) Quél., *Poria incrassata* (Berk. and Curt.) Burt, and other rhizomorphic fungi, they are, as Duggar (*3*) first recognized, "more or less sclerotial and not only serve to spread the fungus vegetatively but are doubtless an important factor in the persistence of the disease in the soil." They also exhibit great diversity in size and structure, which may govern their longevity in the soil. In this connection, it appears highly probable that strands having an entirely cellular structure similar to that of mature sclerotia may persist in the soil for much longer periods than those showing a reduced cortical layer, together with a more or less continuous hollowness of the central portion.

MORPHOLOGICAL STUDIES OF THE SCLEROTIAL STAGE

As shown by other investigators (*6*), the sclerotia or hold-over bodies develop as simple white elongate swellings of the strands of the fungus (pl. 6, *A*), and maximum growth is attained within 10 to 14 days, the fully developed sclerotia being round to ovoid, ellipsoid, variously constricted, and often flattened around the strand connections. With age they change from white to light yellow, and finally to reddish brown. As a rule the individual sclerotia average about 2 mm in diameter, though clusters or nodular masses frequently develop in culture and attain large size, often 10 mm and sometimes 35 mm in diameter. King and Loomis (*6*) state that sclerotia are formed by the division and growth of the cells of portions of the large strands and that this division apparently takes place in the large central hyphae of the strands, as well as in the smaller cells which surround them, as no continuous large cells similar in appearance extend through the sclerotia. In the case of clusters of sclerotia many of the units are developed, by a budding process, directly from the walls of the sclerotia, as well as from the strands themselves.

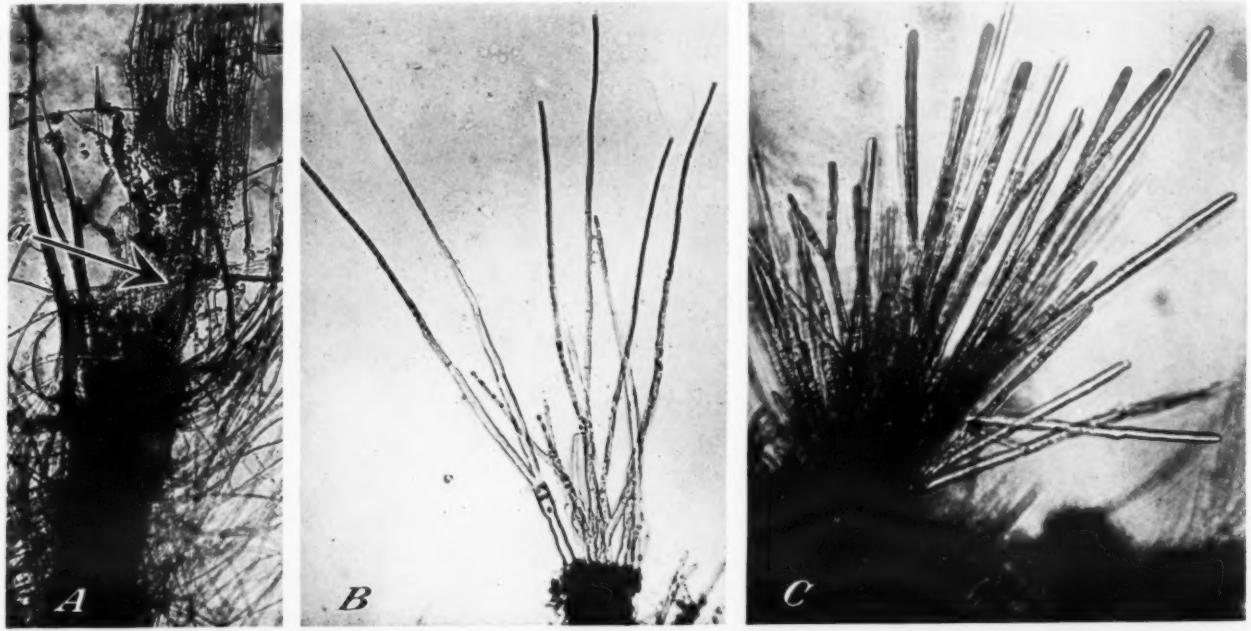
Microscopic examinations of sections of sclerotia obtained from cultures of different ages and from the soil in Texas have largely confirmed the foregoing observations, with certain exceptions when young material was studied. In sections of newly formed sclerotia or of those immersed in the killing and fixing agent just as swelling of the strands begins (usually from 5 to 8 days after the culture is prepared) (pl. 6, *B*), the large central hypha can still be distinguished extending through the sclerotium. The subsequent enlargement and development of the latter is mainly due to the branching and division of the cells of this central mass (pl. 7, *A*). In studies of sections of sclerotial clusters, the budding process mentioned by King and Loomis (*6*) has been frequently observed and appears to be initiated by the tier of cells forming the cortical layer (pl. 7, *B*).

A, Longitudinal section of a young *Phymatotrichum omnivorum* sclerotium (8 days old) showing cellular structure. Note large central hypha extending through the sclerotium. ×335. *B*, Longitudinal section of a sclerotial cluster showing budding of the cortical cells (indicated by arrow), giving rise to a new sclerotium. ×140.



LONGITUDINAL SECTIONS OF PHYMATOTRICHUM OMNIVORUM SCLEROTIA OBTAINED FROM THE SOIL OF INFESTED COTTON PLOTS AT GREENVILLE AND SAN ANTONIO, TEX., DURING JULY 1929, FOLLOWING 2-YEAR GRAIN ROTATIONS.

A, From Greenville. The strand connection is designated by an arrow. ×135. *B*, From San Antonio. ×335. Note cellular structure, with layers of smaller thick-walled epidermal cells and the thin-walled irregular-shaped parenchymalike cells toward the center.



A, *Phymatotrichum omnivorum* strand obtained in July 1931 beneath a primary center of infection in a cotton plot following a 4-year fallow period, showing germination after being in damp chamber for 24 hours. Note vigorous growth of new hyphae (shown at *a*) at the end of the strand. ×100. *B*, Section of strand recovered in August 1932 from an infested plot kept fallow previously for 10 months, showing germination after being in a hanging-drop culture for 16 hours. ×135. *C*, Strands recovered from the soil underlying conidial mats, showing vigorous growth of new hyphae after being in hanging-drop culture for 12 hours. ×135.

STRANDS OF PHYMATOTRICHUM OMNIVORUM RECOVERED FROM THE SOIL BENEATH A CONIDIAL MAT.

Note covering of acicular branches and knotty twisted appearance. ×15.

The fully developed sclerotia, both those occurring in nature and those developing in soil cultures, reveal a distinctly cellular structure, usually with 2 or 3 layers of thick-walled epidermal cells, whereas the cells toward the center are parenchymalike, with thin walls of irregular shape (pl. 8).

HYPHAL STRANDS AS AN OVERWINTERING STAGE

During recent years considerable information has been accumulated by several workers on the importance of the strands of the root-rot fungus as an overwintering stage of the disease. In this connection the views of Duggar (*3*) have already been mentioned, together with the work of Dana (*2*), who reported investigations connecting the appearance of root rot with strands of the fungus found in the soil as the source of infection and demonstrated the power of these strands to produce infection when cotton plants were inoculated. Ratliffe (*15*) also obtained new growth of the fungus from strands found in excavations made beneath spore mats in July 1927; during the season of 1929–30, Neal and McLean (*11*) recovered strands of the fungus from various depths in the soil as late as December 10 and established their viability by germination tests in the laboratory. Taubenhaus and Ezekiel (*19*) also state that the disease may overwinter as dormant strands. More recently Ratliffe (*16*) made excavations in persistent, isolated, carry-over root-rot spots in plots which were planted to cotton only once every 4 years, and found viable strands of the fungus in these areas on dead roots of native trees which had been removed several years earlier. A large number of these strands, cultured by L. G. McLean at the United States San Antonio Field Station, were found to be highly viable. Pure cultures were obtained from the germinating strands and later were successfully used to inoculate cotton plants growing in pots in the greenhouse.

VIABILITY TESTS

During July and August 1931 and 1932, the importance of strands of the fungus in the overwintering of the diseases in the soil was the subject of further studies by the writers. In July 1931, coarse brown strands of the fungus were recovered from an infested cotton plot beneath primary centers of infection at soil depths ranging from 10 to 20 inches. This plot had previously been kept fallow for 4 years. The strands which were recovered occurred in association with old cotton roots and decayed fragments of tree roots below the plow sole, and germinated readily when incubated for 24 hours in damp chambers (pl. 9, *A*). Viable strands were also obtained in August 1931 from a plot that was planted in sorghum but had been planted in cotton the year previous. When the sorghum was harvested and the stubble plowed up, numerous strands that were found intermingled with the sorghum roots were proved to be viable through germination tests. During August 1932, quantities of strands were recovered from a portion of an infested area which had previously been kept fallow for 10 months but which was planted to cotton the previous year. These strands occurred on old much-decayed cotton roots at a soil depth of approximately 10 inches. Sections of some of these strands were placed in hanging-drop cultures and incubated at room temperature for intervals ranging from 6 to 48 hours. In the majority of

cases, they developed a vigorous growth of large-celled hyphae from the ends (pl. 9, *B*), which left no question as to their viability. Some of the strand sections showed germination after being in hanging-drop cultures for only 4 to 6 hours. For the most part, this new growth appeared to originate from the central hypha or from the cells immediately surrounding it.

The writers have made similar tests of the viability of strands in soil cultures and of others associated with the conidial stage of the fungus. It was found that strands obtained from soil cultures and kept in the laboratory for 3 and 8 months, respectively, germinated readily, developing an abundance of hyaline large-celled filaments after being in hanging-drop cultures from 12 to 24 hours. This was also true of the coarse strands underlying conidial mats. Early in September 1932, during a period of wet weather, the conidial stage of the fungus developed rather abundantly in portions of infested cotton plots at Greenville, Tex., affording an opportunity to study the viability of these particular strands. Approximately 10 days after the spore mats had ceased growing, strands pervading the soil beneath the conidial stroma were removed to the laboratory and sections of them were placed in hanging-drop cultures. Examination of these cultures after 12 and 24 hours also revealed a vigorous growth of new hyphae from the ends of the strand sections (pl. 9, *C*). The growth was very similar to that originating from the other subterranean strands described above. It is evident, therefore, that the strands associated with conidial fructifications may retain their viability for some time, even after the spore mats have ceased enlarging, notwithstanding the fact that these particular strands occur very near the soil surface. This is in accord with similar studies previously reported by Neal and McLean (*11*).

For a number of years tests have also been made of the viability of the fungus occurring on the roots of infected cotton plants. Strands were collected during the summer from freshly wilted plants and from those that had been killed by the fungus 3 or 4 weeks previously. The strands obtained from the freshly wilted plants were usually white or light yellow and although of a much finer texture than those obtained from plants killed earlier, they germinated readily after being in hanging-drop cultures from 6 to 12 hours. It was observed, however, that these young strands lost their viability quickly, many failing to germinate if not placed in the hanging-drop cultures almost immediately after they were collected. The older brown strands of coarser texture collected from the roots of plants which had died 3 or 4 weeks before failed to germinate when subjected to similar tests, although several tests were made. The failure to obtain new growth from these strands is in agreement with reports of other investigators (*6*, *11*); the fact that they were not viable, however, is difficult to explain in view of their close morphological similarity to strands growing in culture and to those found at greater depths in the soil. This may be partly due to the close proximity of these strands to the surface of the soil, with the resultant effects of heat and dryness.

This assumption appears to be borne out by the fact that similar strands collected later in the winter [3] from the roots of infected plants on the outer margins of root-rot spots were practically 100 percent

[3] Strands collected Dec. 9, 1932, and Jan. 18 and 30, 1933, respectively, from fields near Garland, Greenville, Floyd, and Dallas, Tex.

viable when tested in hanging-drop cultures. These plants had also been exposed to temperatures ranging from 7° to 8° F. for a few days before they were collected.

It is admitted by most investigators that the root-rot strands are often difficult to find in the soil during the winter months. Neal and McLean (*11*) made numerous excavations in infested cotton fields at several Texas localities during the winter of 1930, but were unable to find the strands of the fungus. The failure to recover them during the winter months of that year might be attributed to the extremely low temperatures that prevailed (minima being recorded which were the lowest in the State for the past 30 years) together with the high moisture content of the soil, which probably caused disintegration of the strands. Notwithstanding these negative results, however, there is some evidence that the strands of the fungus are of importance in the persistence of the disease in the soil, especially in view of the numerous positive tests of their viability on infected roots during the winter of 1932–33, and the further demonstration of their viable nature after prolonged periods in cultures, in fallow plots, and in areas planted to nonsusceptible crops (*8*).

PURE-CULTURE ISOLATIONS FROM HYPHAL STRANDS

As previously stated, pure cultures of the fungus, obtained by Ratliffe and McLean from strands found in the soil on dead tree roots in January 1932, were used successfully to inoculate cotton plants. On July 19, 1932, the writers recovered both bald strands and strands with acicularly branched hyphae beneath primary centers of infection in cotton plots that had been previously kept in clean fallow for 5 years. These strands were removed to the laboratory and of each type 10 that appeared vigorous and apparently viable were used for pure-culture isolations. They were washed thoroughly in distilled water and placed in 1:1,000 bichloride of mercury and 50 percent alcohol for 10 and 2 seconds, respectively. They were then given a second washing in sterile water and placed on corn-meal agar in Petri dishes. After they had been incubated for 48 hours at 29° C., growth developed from the ends of one of the bald strands, which proved to be a vigorous culture of the fungus when transferred to neutral carrot agar. No other pure cultures were obtained, the majority of the strands failing to grow and others developing contaminations.

During the early part of September 1932, a number of coarse brown strands were recovered from the soil beneath several conidial mats which had formed a week or 10 days previously. These underlying strands were entirely fuzzy or acicular and noticeably different from other strands, being knotty and twisted as the result of frequent branching (pl. 10). Cultures were also made of these strands on corn-meal agar after the preliminary disinfecting and washing, as previously described. Out of 20 strands cultured, 1 pure culture was obtained after a 4-day period of incubation. The growth of this culture was very slow and many transfers from the original culture failed to grow. After many attempts, however, and by means of media which had been carefully standardized, viable subcultures were obtained.

INFECTION TESTS

On August 23, 1932, several healthy cotton plants growing in an area free from root rot were inoculated with pure cultures of *Phymatotrichum omnivorum* isolated from the strands collected from the 5-year fallow plot. Twelve days later the inoculated plants developed infection, and after 30 days some of the plants were killed by the fungus. On October 16, the fungus was reisolated from the root of the inoculated plants and later identified microscopically as *Phymatotrichum omnivorum*.

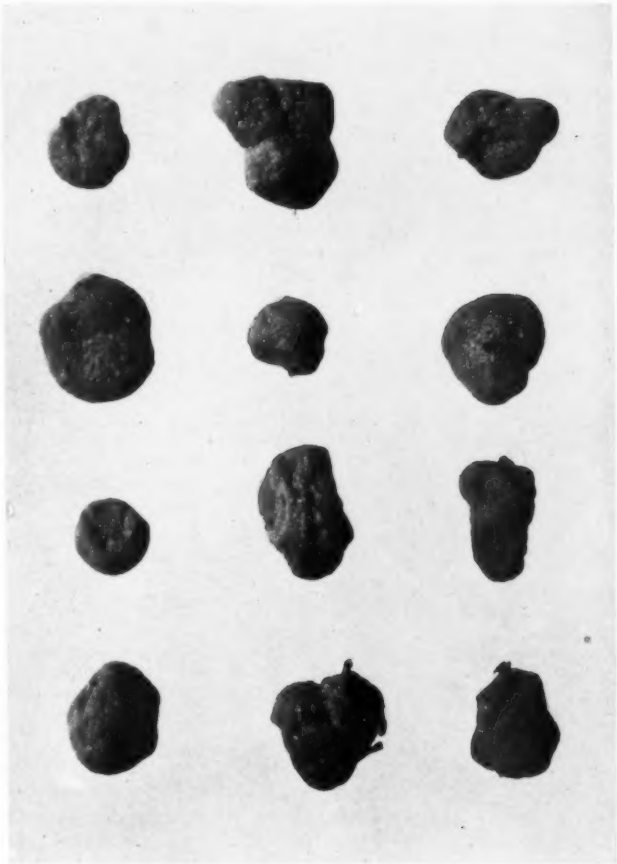
The pathogenicity of the pure cultures obtained from the strands associated with the conidial stage was not established, as the season was too advanced to inaugurate infection experiments with cotton plants under field conditions.

OVERWINTERING IN SCLEROTIAL STAGE

The importance of the sclerotia as an overwintering stage of the disease in Texas, now generally recognized, was first reported in 1929 (*9*). During the spring and summer of that year, primary centers of infection in cotton fields following 2-year grain rotations at Greenville and San Antonio, Tex., were found to be traceable to sclerotia. The sclerotia were recovered beneath wilted plants in large numbers and their viability was established by laboratory tests. Later, during the same year, Dana (*2*) confirmed these observations and found that in cotton plots fallowed previously for 18 months primary centers of root-rot infection which developed originated from sclerotia. Other investigators (*4*, *7*, *18*, *19*, *20*) have also reported finding sclerotia under field conditions in Texas, Arizona, and California, and the ability of these hold-over bodies to produce infection has been amply demonstrated by both greenhouse and field inoculations of cotton plants.

In the majority of soil examinations made by the writers in widely separated Texas localities, extending from the Oklahoma line to the lower Rio Grande Valley, it was found that sclerotia occurred more abundantly in primary centers of infection in areas following grain rotations or long fallow periods. In these centers of infection many of the sclerotia were dark brown to jet black, the outer rind being noticeably tough and difficult to section as compared to that of other sclerotia produced in laboratory cultures (pl. 11). Moreover, in most cases the acicular branches, which usually protrude from the surface cells of younger sclerotia, were entirely lacking, indicating that some of these sclerotia had formed in the soil at the time of the last cotton planting and had remained in a viable condition during the intervening period of grain rotation or fallow treatment.

The mycelium developing from the old germinating sclerotia or even from freshly wilted plants in the above-mentioned centers of infection has a strong tendency to develop almost immediately into new white sclerotia, the growth being practically identical with the elongate swellings of the strands in soil cultures just before the sclerotia are fully developed. Occasionally, the young sclerotia were found attached to young roots in more or less continuous chainlike arrangement in the strands. In areas planted continuously to cotton, however, in which large proportions of the plants are killed by the disease, the sclerotia are always difficult to find, a fact which suggests



SCLEROTIA OF PHYMATOTRICHUM OMNIVORUM RECOVERED FROM THE SOIL OF AN INFESTED COTTON PLOT IN A PRIMARY CENTER OF INFECTION AT SAN ANTONIO, TEX., JULY 19, 1932, FOLLOWING A 2-YEAR GRAIN ROTATION.

Ninety percent of these sclerotia were viable. ×8.

that under these conditions the subterranean strands, which are in reality elongate sclerotia, undoubtedly serve to perpetuate the disease in the soil.

SUMMARY

Studies of the morphology of the ozonium and sclerotial stages of the cotton root-rot fungus, *Phymatotrichum omnivorum*, are reported, together with information bearing upon the life cycle of the fungus in the infested districts of Texas. The hyphae involved in the growth and development of the fungus are described, as well as the type concerned in the penetration of the host tissues. Comparisons are given of the structure of the strands and sclerotia at various stages of their development. Studies of the strands included histological preparations of subterranean strands, strands obtained from soil cultures, and strands underlying conidial mats. The cellular structure of both young and old strands, as shown in longitudinal and cross sections, was found to be quite diverse. The central portion was in some cases well defined, comprising from 1 to 3 large septate hyphae, while in others the strands were entirely cellular, revealing in general the morphology of true sclerotia. The sclerotial character of the strands show that they function not only to spread the fungus vegetatively but also to maintain it in a viable condition during prolonged periods of inactivity. In this respect, they are analogous to the rhizomorphs of certain Basidiomycetes.

Histological studies of mature and newly formed sclerotia obtained both from laboratory cultures and from the soil have largely confirmed the work of other investigators. In examinations of sections of young sclerotia it was found that the large central hypha extends through the sclerotium and that it is largely as a result of the branching and division of these cells that the sclerotia are formed. The gross morphology and cellular structure of some of the sclerotia found in the infested districts of Texas are described.

The importance of the strands in the overwintering of the disease is further shown by the numerous positive tests of their viability under varying conditions, especially those from fallow plots, from areas planted to nonsusceptible crops, and from soil cultures after prolonged intervals. The ability of these strands to produce infection was further demonstrated by pure-culture isolations and inoculation experiments.

The importance of the sclerotial stage of the fungus in the perpetuation of the disease in the soil is also discussed, and additional information given regarding the occurrence of this stage in nature.

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EFFECT OF MILDEW AND RUST INFECTION ON DRY WEIGHT AND RESPIRATION OF EXCISED CLOVER LEAFLETS[1]

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INTRODUCTION[3]

In the course of a study of mildew (*Erysiphe polygoni* DC.) and rust (*Uromyces fallens* (Desm.) Kern) of red clover (*Trifolium pratense* L.), the effect of these parasites on the dry weight and respiration of clover leaves was determined, since it was believed that such information might be of value in explaining the nature of the injury caused by these fungi. The triplet-leaflet method previously described[4] made possible the use of small but closely comparable units of leaflets in which dry-weight changes and respiration could be accurately measured.

MATERIALS AND METHODS

Leaves of red clover from mildew- and rust-susceptible greenhouse plants served as host material. For inoculum, urediospores of clover rust and conidia of clover mildew were produced on excised clover leaflets floating on sucrose solution. Leaflets were inoculated with mildew by touching or dusting the upper (ventral) surface of the leaflet with a heavily sporulating mildew colony produced on another leaflet. In the rust inoculations urediospores were dusted from an infected leaflet on to the lower (dorsal) surface of the healthy leaflet and water was atomized on the inoculated surface.

Groups of leaves selected for a given experiment were each divided into three strictly comparable units by placing one leaflet from each leaf into each of three containers, i.e., flasks or Petri dishes. An experimental unit consisted of 6 to 12 leaflets, the number depending on the size of the leaflets and of the container. Usually one unit of leaflets from each group of leaves served as a control, one was inoculated with mildew, and the third was inoculated with rust. After 12 days the mildew and rust were sporulating heavily and the leaflets were washed to free them of sucrose, dried at 102° C. for 4 days, and weighed. The weight of the mildew and rust spores applied as inocu-

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[2] The writer wishes to acknowledge his indebtedness to Dr. M. W. Gardner and Dr. R. M. Caldwell, of the Purdue University agricultural experiment station, to Dr. R. E. Girton, of Purdue University, and to Dr. E. A. Hollowell, of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, for assistance in the course of this work and in the preparation of the manuscript.

[3] At the time of conducting these investigations and preparing this paper, the writer was unaware of the following publication: LOBIK, A. T. ON THE QUESTION OF THE INFLUENCE OF PARASITIC FUNGI ON CLOVER CROPS. Plant Diseases, Jour. Centr. Phytopath. Stat. Chief Bot. Gard., Russian Republic, 2-8. 1922. [In Russian. Reviewed in Rev. Appl. Mycol. 3: 40. 1924.] In this paper the following statement is made: "In the majority of cases *Uromyces trifolii* was observed to stimulate the vegetation of the plants attacked resulting in a heavier production both of green parts and of seed."

[4] YARWOOD, C. E. THE COMPARATIVE BEHAVIOR OF FOUR CLOVER LEAF PARASITES ON EXCISED LEAVES. Phytopathology 24: 797—806, illus. 1934.

lum and the weight of the spores lost in washing the leaflets were not considered. Percentage dry weights were calculated from the final dry weight and the original green weight of each experimental unit, since preliminary tests had shown that percentage dry weight was the most uniform basis of comparison, the standard deviation of three units from the same group of leaves being 1.65 ± 0.227 percent. It was assumed that percentage dry weights of the three units of leaflets from the same group of leaves were originally equal and that the final differences were due to the experimental treatments. This assumption is subject to the error shown by the standard deviation given above.

In order to interpret the effect of mildew and rust infection on the dry weight of excised clover leaflets floating on sucrose solution, it was necessary to determine the effect on dry weight of floating uninoculated leaves on the nutrient solution for the 12-day period allowed in these studies for disease development. A test of six units was therefore made which showed that there was a mean increase of 2.8 ± 1.78 percent of the original dry weight due to floating the leaflets on their dorsal surface on 10-percent sucrose.[5] This increase in dry weight is small and is not statistically significant, since the probable error of the determination is large. A further test was made to determine the effect on the final dry weight of floating the leaflets with their ventral surfaces on the sucrose solution. This test revealed that the dry weight was 13.2 ± 0.66 percent greater than when the leaflets were floated on their dorsal surfaces. This emphasizes the importance of handling all experimental units in an identical manner, and unless otherwise mentioned the excised leaflets were all floated with their dorsal, hairy surfaces on the solution.

Respiration experiments were conducted on the same general plan as were the studies on dry-weight changes, i.e., by the triplet-leaflet method of assembling groups of three comparable units of leaflets. Simultaneous determinations could thus be made of the respiration of one unit of leaflets inoculated with mildew, a second unit inoculated with rust, and a third uninoculated control unit.

Respiration of leaflets in 250-cc flasks was measured by two methods, namely, gas analysis and the use of manometers. For gas analysis each flask was fitted with an outlet tube and stopcock. The amount of oxygen (O_2) and carbon dioxide (CO_2) in samples from each flask was measured daily or at 2-day intervals by means of a Haldane-Henderson-Bailey gas-analysis apparatus.[6] The weight of CO_2 produced by respiration at the end of any given period was determined by the formula

$$w = b\left(\frac{ctv^1}{t^1} + cv^2v^3\right) + w^1,$$

where

w = weight in grams of CO_2 produced by respiration at end of any given period.

b = weight of 1 cc of CO_2 at normal temperature and pressure = 0.001976 g.[7]

[5] In this same test there was a mean decrease of 15 ± 1.1 percent in dry weight due to floating the leaflets on their dorsal surfaces on water for 12 days.

[6] BAILEY, C. V. APPARATUS USED IN THE ESTIMATION OF BASAL METABOLISM. Jour. Lab. and Clin. Med. 6: 657-679, illus. 1921.

[7] HODGMAN, C. D., and LANGE, N. A. HANDBOOK OF CHEMISTRY AND PHYSICS; A READY-REFERENCE POCKET BOOK OF CHEMICAL AND PHYSICAL DATA. Ed. 13, 1214 pp. Cleveland, Ohio. 1928.

c=fractional part of CO_2 in enclosed gas minus fractional part of CO_2 in air.
t=273° Abs.
t^1=temperature of experiment=296° Abs.
v^1=volume in cubic centimeters of gas space in flask.
v^2=volume of CO_2, at normal temperature and pressure, dissolved in 1 cc of water at temperature of experiment=0.804 cc.
v^3=volume of sugar solution in flask=25 cc.
w^1=weight in grams of CO_2 removed in previous samples.

These calculations are based on the assumptions that the barometric pressure was constant at 760 mm and that the solubility of CO_2 in 10-percent sucrose is the same as its solubility in water. It is believed that these assumptions do not introduce errors of serious consequence to this study. The weight in grams of CO_2 removed in previous samples (w^1) was secured by adding together the weights of CO_2 removed in each previous sample from the same flask. The weight of CO_2 removed in a single sample for gas analysis was determined by the formula

$$w^2=\frac{cv^4tb}{t^1},$$

where
w^2=weight in grams of CO_2 in a single sample.
v^4=volume in cubic centimeters of sample.
c, t, t^1, and b signify the same values as in the preceding formula.

The respiratory quotient ($r\,q$) was determined by dividing the volume of CO_2 evolved during respiration by the volume of O_2 utilized.

The manometric method of measuring respiration was essentially the same as that described by Girton [8] except that 250-cc flasks were used as containers, the respiratory materials (clover leaflets) were floated on 10-percent sucrose in the flask, and the CO_2 evolved was absorbed in a vial of concentrated potassium hydroxide (KOH) suspended from the stopper of the flask. A respiration flask with leaves and manometer is shown in figure 1. The amount of CO_2 produced in a manometer flask at the end of any given period was determined by the formula

$$w=\frac{bmtvr}{t^1p},$$

where
w=weight in grams of CO_2 produced by respiration at end of any given period.
b=weight of 1 cc CO_2 at normal temperature and pressure=0.001976 g.[9]
m=manometric reading (in millimeters of mercury).
t=273° Abs.
v=volume in cubic centimeters of gas in flask.
r=average respiratory quotient as measured by gas analysis=0.867.
t^1=temperature of experiment=294° Abs.
p=760 mm (normal atmospheric pressure).

[8] GIRTON, R. E. A SIMPLE RESPIROMETER FOR QUANTITATIVE CLASS EXPERIMENTS. Ind. Acad. Sci. Proc. (1929) 39: 119–122, illus. 1930.
[9] HODGMAN, C. D., and LANGE, N. A. See footnote 7.

This formula is based on the assumptions that the changes in atmospheric pressure during the experimental period were negligible and that the respiratory quotient was the same as in the gas-analysis determinations, i.e., 0.867. The temperature was about 23° C. for the gas-analysis experiments and about 21° for the measurements by manometers.

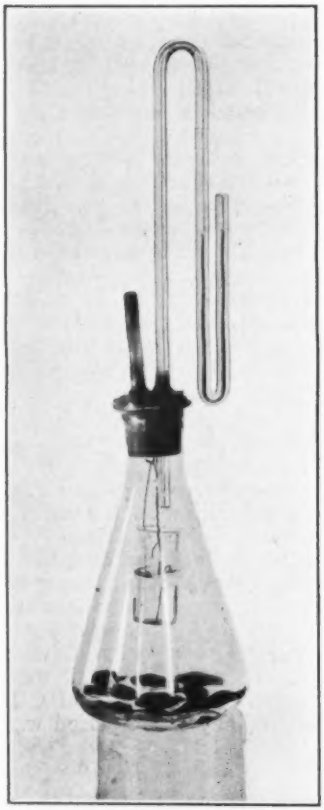

FIGURE 1.—Flask fitted with manometer, for the measurement of respiration. KOH in vial suspended from cork absorbs CO_2 formed by respiration. Amount of O_2 utilized in respiration is determined from change in internal pressure, as measured by change in mercury level in manometer.

In the respiration studies the green weight of the leaflets was not determined, and any changes in dry weight were determined from the final dry weights. This involves the assumption that the final dry weight of uninoculated leaflets floated on 10-percent sucrose is not significantly different from the original dry weight of these same leaflets, as was concluded from results given earlier.

In these experiments the leaflets were floated on 25 cc of 10-percent sucrose solution in 250-cc flasks held in the darkness, and no attempt was made to maintain aseptic conditions. The sugar solutions, however, remained clear during the experimental period, indicating that there was little development of contaminating organisms, and it is believed that the respiration measured was almost entirely that of the healthy or infected leaflets.

EXPERIMENTAL RESULTS

DRY WEIGHT OF MILDEWED AND RUSTED LEAFLETS

The effect of mildew and rust infection on dry weight of the clover leaflets was measured in a variety of experimental treatments and was calculated from the differences between the percentage dry weights of uninfected units of leaflets and those of similar mildew and rust-infected units.

The mean and the probable error of the differences in the final percentage dry weights were determined by taking differences between paired values directly.[10] All experiments to determine the effect of mildew and rust on dry weight are summarized in table 1. In all cases the mildew-infected leaflets were considerably lower and the rust-infected leaflets were considerably higher in dry-matter content than the check leaflets on sucrose solution.

[10] HAYES, H. K., and GARBER, R. J. BREEDING CROP PLANTS. Ed. 2. 438 pp., illus. New York. 1927.

TABLE 1.—*Effect of mildew and rust on dry weight of excised clover leaflets*

Treatment	Replications	Decrease due to mildew	Increase due to rust
	Number	*Percent*	*Percent*
All leaflets floated on dorsal surface in covered Petri dishes	5	9.85±0.95	8.42±1.56
All leaflets floated on ventral surface in covered Petri dishes	4	11.61±1.15	2.74±1.96
All leaflets floated on dorsal surface in closed 250-cc flasks [1]	4	6.88±1.88	11.86±1.35
All leaflets floated on dorsal surface in closed 250-cc flasks; CO_2 absorbed by KOH solution [1]	4	7.84±2.99	17.29±5.14
Average		9.04±1.74	10.07±2.50

[1] The same experimental units were used in the respiration studies.

As might be expected from the results with healthy leaves, the decrease in dry weight due to mildew infection was greater and the increase due to rust infection was less when the leaflets were floated with their ventral surface on the solution than when they were floated with their dorsal surface on the solution. For some reason which the writer cannot explain, the decrease in dry weight due to mildew infection was less and the increase in dry weight due to rust infection was greater for the leaflets in flasks than for those in Petri dishes.

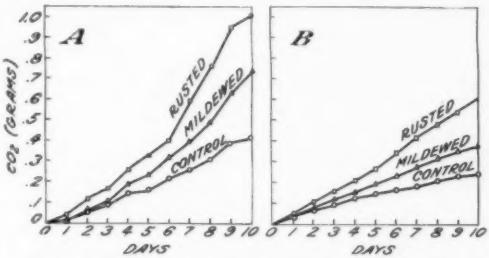

FIGURE 2.—Effect of mildew and rust infection on respiration of clover leaflets, expressed as grams of CO_2 produced per gram of dry weight of respiring leaves: *A*, Data as determined by manometers; *B*, data as determined by gas analysis.

RESPIRATION OF MILDEWED AND RUSTED LEAFLETS

The respiration of controls and of mildewed and rusted leaflets was studied in four groups by manometers and in four groups by gas analysis. One group of manometer flasks leaked and gave unsatisfactory results. The actual data as determined by the formulas given were converted into grams of CO_2 per gram of final dry weight of leaf tissue in the check unit. The results are given in table 2 for the manometer series and in table 3 for the gas-analysis series. The results are plotted graphically for one group of determinations by manometers in figure 2, *A*, and for one group of gas analyses in figure 2, *B*. The respiration curves determined by the gas-analysis method were quite smooth, whereas those from the manometer readings were rather irregular. For the period of observation, which in these experiments was 9 to 11 days, mildew increased respiration on an average for all tests by 41 percent, and rust increased respiration by 123 percent.

The average rate of respiration measured as grams of CO_2 per gram of dry weight of leaf tissue during a 9-day period was 0.265 g CO_2 for the control leaflets, 0.369 g CO_2 for the mildewed leaflets, and 0.580 g for the rusted leaflets.

TABLE 2.—*Effect of mildew and rust infection on respiration of excised clover leaflets, as determined with the manometer*

Time from sealing of flasks	Grams of CO_2 per gram of dry weight of leaves of indicated group and condition								
	Group 1			Group 2			Group 3		
	Control	Mildewed	Rusted	Control	Mildewed	Rusted	Control	Mildewed	Rusted
1 day	0.0064	0.0108	0.041	0.020	0.014	0.026	0.020	0.011	0.025
2 days	.055	.059	.119	.104	.088	.056	.090	.006	.119
3 days	.087	.108	.161	.111	.144	.008	.111	.137	.198
4 days	.147	.190	.265	.167	.231	.163	.158	.228	.318
5 days	.156	.236	.333	.208	.231	.292	.192	.273	.422
6 days	.220	.321	.505	.177	.238	.335	.173	.285	.461
7 days	.256	.392	.593	.249	.286	.481	.237	.393	.568
8 days	.307	.489	.755	.281	.308	.563	.265	.465	.657
9 days	.394	.631	.946	.284	.318	.631	.271	.506	.715
10 days	.412	.739	1.030						

TABLE 3.—*Effect of mildew and rust infection on respiration of excised clover leaflets, as determined by gas analysis*

GRAMS OF CO_2 PER GRAM OF DRY WEIGHT OF LEAVES OF INDICATED GROUP AND CONDITION

Time from sealing of flasks	Group 1			Group 2			Group 3			Group 4		
	Control	Mildewed	Rusted	Control	Mildewed	Rusted	Control	Mildewed	Rusted	Control	Mildewed	Rusted
1 day	0.0386	0.0478	0.0545									
2 days	.0745	.0847	.1123	0.0436	0.0531	0.0703						
3 days	.0940	.1252	.1633	.0799	.0958	.1155	0.0818	0.0975	0.1206	0.0875	0.1060	0.1062
4 days	.1352	.1590	.2150	.1130	.1158	.1425						
5 days	.1492	.2007	.2745	.1338	.1478	.1880	.1260	.1510	.2250	.1218	.1413	.1762
6 days	.1695	.2370	.3480	.1590	.1670	.2288						
7 days	.1830	.2830	.4245	.1832	.1925	.2950	.1807	.2260	.3332	.1693	.1998	.2260
8 days	.2147	.3225	.4875	.2080	.2162	.3577						
9 days	.2370	.3567	.5510	.2308	.2483	.4120	.2300	.2700	.4852	.2086	.2535	.3222
10 days	.2455	.3810	.6170	.2744	.2680	.4620						
11 days				.2650	.2819	.5210	.2790	.3365	.6070	.2418	.3122	.3835

FINAL CONCENTRATION OF CO_2 IN FLASKS AT LAST ANALYSIS (PERCENTAGE BY VOLUME)

10 days	4.44	6.61	10.84									
11 days				4.18	4.75	9.02	5.32	6.60	11.64	5.73	7.14	9.00

EFFECT OF AERATION ON RESPIRATION OF RUST-INFECTED LEAFLETS

The respiration rates for the controls and for mildewed and rusted leaflets were greater as determined by manometers than by gas analysis. This difference may have been caused by the accumulation of CO_2 in the gas-analysis flasks, which became as high as 12 percent of the volume of the enclosed gas and which may have acted as a check on the respiratory process. No such accumulation of CO_2 occurred in the manometer flasks, where it was absorbed by the KOH solution. At the end of the experimental period the rusted leaflets from the gas-analysis flasks were sporulating less heavily than the rusted leaflets from the manometer flasks. This may have resulted from the accumulating CO_2. To determine whether the high concentration of CO_2 inhibited respiration, the effect of aeration on respiration was tested by gas analysis, with two groups of clover

leaves. One unit of each sample served as a control; the two others were inoculated with rust. One of the latter flasks in each group was aerated at the time of sampling the enclosed gas, while the other remained closed except to permit the removal of the 10-cc sample required for gas analysis. In this experiment the CO_2 content of the enclosed air was measured every 2 days, and aeration was started on the fourth day. At each aeration the gas in the flask was brought to the composition of laboratory air by aspirating the flask with a suction pump for about 2 minutes. The respiration determinations for the duplicate groups are given in table 4, and the results for one group are illustrated in figure 3. The respiration of the aerated leaflets began to rise immediately after aeration was started. Even this degree of aeration in the aerated flasks did not reduce the CO_2 concentration to as low a point as that in the manometer flask or in the natural environment, since the CO_2 content of the aerated flasks rose to 3 percent during the 2-day periods between analysis. The results, however, indicate an inhibition of respiration due to a high concentration of CO_2.

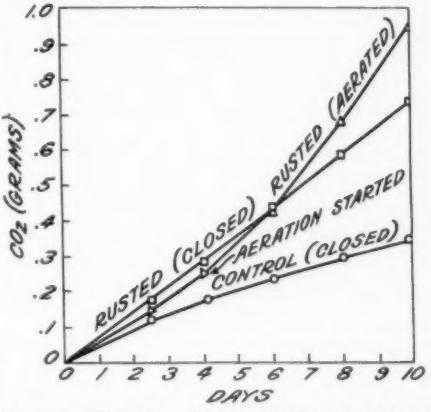

FIGURE 3.—Effect of aeration on respiration of rust-infected clover leaflets, expressed as grams of CO_2 produced per gram of dry weight of respiring leaflets.

TABLE 4.—*Effect of aeration on respiration of rust-infected clover leaves*

GRAMS OF CO_2 PER GRAM OF DRY WEIGHT OF LEAVES OF INDICATED GROUP AND CONDITION

Time from sealing of flasks	Group 1			Group 2		
	Control	Rusted closed	Rusted aerated [1]	Control	Rusted closed	Rusted aerated [1]
2½ days	0.1187	0.1752	0.1448	[2] 0.1220	[2] 0.1592	[2] 0.1362
4 days	.1714	.2880	.2475	.1674	.2762	.2146
6 days	.2333	.4365	.4290	.2298	.4660	.3835
8 days	.2925	.5897	.6795	.3085	.7180	.7215
10 days	.3452	.7320	.9600	.3447	.7835	.9250
FINAL CONCENTRATION IN CO_2 IN FLASKS AT LAST ANALYSIS (PERCENTAGE BY VOLUME)						
10 days	5.19	11.02	3.21	5.09	12.12	2.59

[1] Aeration started on fourth day.
[2] 3 days from sealing of flasks.

EFFECT OF DUSTING WITH SULPHUR ON RESPIRATION OF HEALTHY AND MILDEWED LEAFLETS

The increased respiration found in mildew-infected leaves might be due to the respiration of the mildew fungus in addition to that of the leaf, or to a stimulated respiration of the leaf as a result of the diseased condition, or to the two combined. If the increased respiration was largely or entirely due to the respiration of the mildew, then the rate of respiration of mildewed leaves would probably decrease if the mildew was killed. This possibility was investigated by killing the mildew with sulphur dust.

Clover leaves that were heavily mildewed on both surfaces were taken from field plants. Two groups of leaves were each divided into two comparable units on the same basis as in the triplet-leaflet method. One unit of each group was placed in a respiration flask without further treatment, and the other unit of each group was heavily dusted with sulphur. It is believed that the mildew was thereby killed, since in a previous experiment, in which vigorous mildewed leaflets were lightly dusted with sulphur and the sulphur dust washed off after 2 days, there was no further development of mildew. The extreme sensitiveness of clover mildew to sulphur has been observed in numerous other experiments.

The leaflets in this experiment were not floated on nutrient solution but were free in dry flasks. As the flasks were stoppered, however, the leaflets did not dry out, but remained turgid and in good condition during the 3-day experimental period. The gas in each flask was analyzed daily. At the end of the period the area of the leaflets was measured and the results were calculated on the unit-area basis, since the dry weight of these groups of leaflets was not determined.

The area of the leaflets was measured by tracing their outlines on squared paper, measuring the outlines by counting the squares, and doubling the measured area to give the actual surface area. The data from the duplicate groups were similar, and are given in table 5. In both cases the sulphured mildewed leaflets show the higher rate of respiration, and the average increase in the respiration of the sulphured units over the controls was 18 percent.

TABLE 5.—*Effect of dusting with sulphur on respiration of healthy and mildewed clover leaves*

Time after treatment	Grams of CO_2 produced per square decimeter of leaf surface					
	Group 1 (healthy leaves)		Group 2 (mildewed leaves)		Group 3 (mildewed leaves)	
	Control	Sulphured	Control	Sulphured	Control	Sulphured
1 day	0.0142	0.0162	0.0212	0.0250	0.0238	0.0271
2 days	.0218	.0252	.0383	.0442	.0378	.0416
3 days	.0261	.0289	.0485	.0607	.0481	.0537

The effect of sulphur dust on the respiration of mildew-free leaves from field-grown plants was tested in the same way and at the same time as were the mildewed leaves in the experiment just described. Only one group of healthy leaves was studied. Here, again, the leaflets dusted with sulphur had the higher rate of respiration. The results showing the effect of sulphur dust on the respiration of healthy and mildew-infected red clover leaflets are given in table 5, and the

results with one group of mildewed leaves and with the single group of healthy leaves are shown graphically in figure 4.

These experiments indicate that the increased respiration of mildew-infected leaflets was mainly due to the stimulation of respiration of the host by the mildew rather than by respiration of the mildew fungus.

RESPIRATORY QUOTIENT

In most cases only the CO_2 content of the gas-analysis samples was measured, but in a number of cases the O_2 content was also measured and the respiratory quotient was determined. The mean respiratory quotient of the check units was 0.855 ± 0.0051 (23 determinations); of the mildewed units, 0.859 ± 0.0190 (22 determinations); and of the rusted units, 0.888 ± 0.0184 (24 determinations). The respiratory quotients of check, mildewed, and rusted units of leaflets were therefore not significantly different.

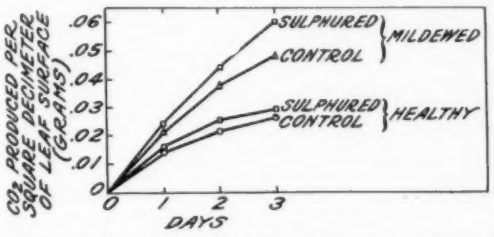

FIGURE 4.—Effect of dusting with sulphur on respiration of healthy and mildewed clover leaflets.

AMOUNT OF SUCROSE ABSORBED FROM NUTRIENT SOLUTION BY MILDEWED AND RUSTED LEAFLETS

Although the data presented do not directly show differences in the absorption of sucrose, it is thought that such differences can be determined approximately. Assuming that only sucrose was used in respiration and that the respiratory quotient was 1, the amount of sucrose utilized can be found from the amount of CO_2 evolved, according to the equation

$$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O.$$

Actually the respiratory quotient was not 1, but about 0.867. For the purpose of this discussion it is assumed that the amount of O_2 in excess of that required to give a respiratory quotient of 1 was utilized in other processes than respiration and that the amount of sucrose absorbed was proportional to the amount of CO_2 evolved. Assuming that the final dry weight of uninoculated leaflets on 10-percent sucrose was the same as their original dry weight, that the sugar absorbed was stored as sucrose (the iodine test showed practically no starch present), and that there was no diffusion from the leaflets, the amount of sucrose absorbed by an experimental unit of leaflets would be equal to the final dry weight of the leaflets minus their original dry weight plus the amount of sucrose utilized in respiration. This method of calculation has been applied to the final data from the gas-analysis experiments, and the results are given in table 6. It appears from such a calculation that rust infection has caused a greatly increased absorption of sucrose, whereas mildew has had little if any effect on sucrose absorption. This may be partly due to the morphological relationships of mildew and rust infection.

TABLE 6.—*Grams of sucrose absorbed per gram of dry weight of clover leaflets during a 12-day period* [1]

Group no.	Check (healthy leaflets)	Mildewed leaflets	Rusted leaflets	Group no.	Check (healthy leaflets)	Mildewed leaflets	Rusted leaflets
1	0.190	0.145	0.599	5	0.222		0.892
2	.197	.154	.554	6	.191		.502
3	.180	.179	.492				
4	.145	.168	.318	Average	.187	.161	.559

[1] Calculated indirectly from dry weight and respiration determination. (See p. 556.)

DISCUSSION

The conditions in dish cultures, under which the foregoing experiments were performed, were so entirely different from the condition of growing plants that it is difficult to correlate the results with those obtained under natural conditions. There is apparently a fundamental difference, however, in the two clover-leaf parasites studied. Under the experimental conditions, mildew caused a decrease in dry weight of the leaflets, whereas rust infection caused an increase. It is not known to what extent the increase in dry weight of rust-infected leaflets is due to the increase in the weight of the leaf tissue by reason of the absorption of sucrose or to what extent it is due to the weight of the rust mycelium.

The differences in the extent of invasion of the leaf by the mildew and rust fungi may bear a relation to these results. Mildew inoculated on one surface of the leaf invaded only the epidermal cells of the inoculated surface, which were separated from the absorbing surface by the leaf parenchyma, and any effect of mildew infection on sucrose absorption would have to be exerted through this parenchyma. With rust infection, on the other hand, the mycelium invades the entire leaf, and at sporulation both the upper and lower epidermis may be ruptured. Thus sucrose absorption might take place more readily after rust infection than after mildew infection.

SUMMARY

Excised clover leaflets floated on sucrose nutrient solutions have been found to be suitable experimental material for the comparison of changes in dry weight and respiration caused by mildew (*Erysiphe polygoni* DC.) and rust (*Uromyces fallens* (Desm.) Kern).

A greater absorption of sucrose resulted when the leaflets were floated with their lower surfaces in contact with the sugar solution than when they were floated with their upper surfaces in contact with the solution.

Infection with mildew caused an average decrease of 9 percent in the dry weight of excised leaflets during a 12-day period, whereas infection with rust caused an average increase of 10 percent in dry weight.

Infection with mildew caused an average increase of 41 percent and infection with rust caused an average increase of 123 percent in the respiration of clover leaflets during the 9- to 11-day period following inoculation.

The rate of respiration of rust-infected leaflets was greater in aerated respiration flasks than in flasks in which the respiratory gases were allowed to accumulate.

Dusting the leaflets with sulphur increased the respiration of both healthy and mildew-infected clover leaflets.

PENETRATION, DISTRIBUTION, AND EFFECT OF PETROLEUM OILS IN APPLE[1]

By PAUL A. YOUNG

Assistant botanist and bacteriologist, Montana Agricultural Experiment Station[2]

INTRODUCTION

The objectives of the project on oil sprays of apple were to determine: (1) The concentrations of oils that apple leaves tolerate without showing necrotic symptoms, (2) the symptoms caused by petroleum oils in tissues, (3) the rates of oil penetration into leaves, (4) the distribution of petroleum oils in apple tissues, and (5) a rapid, easy, accurate method for predicting the probable injury that an oil spray will cause in apple leaves.

In a previous paper Young and Morris (*18*)[3] described the concentrations of oils in emulsion sprays that were tolerated by apple leaves, and the injuries that were caused by sprays containing 2 to 16 percent of oils. The experiments with emulsions fulfilled only the first objective of the project, because emulsions containing 1 and 2 percent of oils rarely caused symptoms in apple leaves, and emulsions containing 1 to 16 percent of oils were inadequate in determining the penetration, distribution, effect, and toxicity of each oil. Hence, undiluted oils were tested on apple leaves to fulfill the last four objectives of the project. Furthermore, sprays with undiluted-oil fogs have important advantages as commercial insecticides. The present paper describes the experiments with dissimilar undiluted oils.

REVIEW OF LITERATURE

Young and Morris (*18*) described the effects of sprays with oil emulsions on apple. They used 19 of the 29 oils described in figure 1 of the present paper and reviewed literature on the relation of viscosity to injury by oil.

Herbert (*9*) described the commercial advantages of spraying fogs of nearly pure oils on deciduous trees.

Young[4][5][6] (*15*, *16*, *17*) described symptoms caused by oils, the parts of tissues containing oils, and freezing phenomena in spray-oil emulsions. He explained theoretically how oils enter protoplasm and showed that *Rhizopus nigricans* Ehr. can be used to indicate the probable effects of oils on apple leaves.

Groves, as reported by Kendall (*11*), found that oil replaced the air in the intercellular spaces of leaves, causing translucence; the oil remained indefinitely in the positions first occupied. English (*2*) wrote

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[2] The writer wishes to thank Professors H. E. Morris, D. B. Swingle, F. B. Cotner, and J. R. Green, of the Montana Agricultural Experiment Station and College of Agriculture, for criticizing this manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. —.

[4] YOUNG, P. A. SPRAY OIL PENETRATION OF APPLE LEAVES, LIMBS AND FRUIT. Abstract of Papers, Amer. Soc. Plant Physiol., Des Moines, Iowa, pp. 11–12. 1929. [Mimeographed.]

[5] ——. OIL-MASS THEORY OF PETROLEUM-OIL PENETRATION INTO PROTOPLASM. Abstract of Papers, Bot. Soc. Amer., Boston, Mass., pp. 11–12. 1933. [Mimeographed.]

[6] ——. FREEZING PHENOMENA IN CRESOAP EMULSIONS OF PETROLEUM OILS. Abstract of Papers, Amer. Soc. Plant Physiol., Boston, Mass., pp. 14–15. 1933. [Mimeographed.]

that pure 7- to 9-percent sulphonatable oils blackened tissues within 2 days, while pure 0- to 3-percent sulphonatable oils slowly caused chlorosis and defoliation. Kelley (*10*) stated that oil emulsions entered the intercellular spaces of leaves. Ginsburg (*3, 4*) determined that oils penetrate apple leaves at rates inversely proportional to the viscosities and directly proportional to the concentrations of the oils.

Bartholomew (*1*) reported that oil entered through both sides of citrus leaves and passed between and into the cells. Knight, Chamberlin, and Samuels (*12*) illustrated oils in and between the cells of orange leaves and stated that oils passed into the phloem, medullary rays, and pith of stems. Proescher (*13*) used Oil Red O, and Haynes (*8*) used Sudan IV for staining oils.

Woglum, LaFollette, Landon, and Lewis (*14*) observed that oil sprays on citrus trees were associated with an increase in dead wood, fruit dropping, culled fruit, and navel water rot.

Harvey (*7*) described the pneumatic and hydrostatic systems in apple, as studied by injected aqueous solutions of dyes, and reviewed literature on this subject.

Green (*6*) found evidence that filtration, sulphonation, and alcoholic extraction removed some of the toxic materials in oils.

MATERIALS AND METHODS

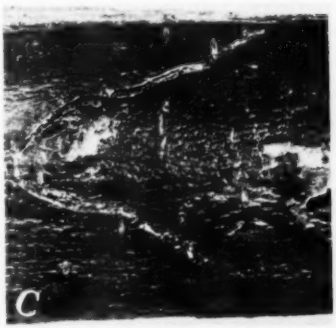
The petroleum oils listed in figure 1 were tested on Hibernal apple in an orchard on the farm of the Montana Agricultural Experiment Station at Bozeman, Mont., during 1926 to 1933. Before using, some samples of oils 3, 13, and 24 were saturated with Oil Red O to make them easily discernible in tissues. Indophenol Blue was used in one sample of oil 24. The oils were painted in undiluted form on apple leaves to determine promptly the effect of each oil. Undiluted oils were used, except where otherwise stated.

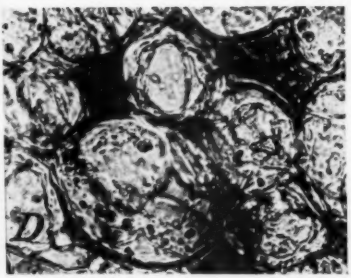
The distribution of oils in apple tissues was determined by sectioning oil-treated leaves and their twigs 1 to 15 cm from the leaves. The tissues were sectioned 1 to 488 (usually 8 to 80) days after the oils were placed on the leaves. Oils were placed on apple fruits, and sections were made later to determine the penetration and distribution of oils in the fruits. The sections were studied with and without staining. Tissues separately containing 10 of the oils were studied on 600 slides. Comparable leaves, stems, and fruits to which no oils had been applied were sectioned, stained, and studied for comparison with the oiled tissues.

The following methods were used in staining oils:

Sections were cut from freshly collected leaves and stems, and were stored in water for a few minutes. They were then (1) placed in equal parts of pure acetone and aqueous solution of 70-percent ethyl alcohol for a few minutes to remove chlorophyll and prepare them for staining, (2) stained for 1 to 3 minutes in a saturated solution of Sudan IV in equal parts of acetone and 70-percent ethyl alcohol, (3) destained for 1 to 3 minutes in the alcohol-acetone mixture, (4) washed for a few minutes in water, (5) counterstained for 1 to 3 minutes in a solution of 10-percent Unna's alkaline methylene blue, (6) washed in water for a few minutes, (7) mounted on slides in pure glycerine, and (8) sealed on the slides with clear Duco varnish.

Tissues were preserved with water and were cut soon after collecting, or were stored in a 60-percent solution of pyridine during 1 to 60





A, Angular mottling (white) representing oil 21 in polyhedrons of mesophyll in an apple leaf. This leaf was excised and dried 8 days after oiling and was photographed 348 days after oiling, showing that the vein-bounded polyhedrons of mesophyll held the oil; 384 days later, only one oily-translucent polyhedron remained in this leaf, showing that most of the oil had disappeared from the other polyhedrons that were occupied by oil when photographed. Irregular brown lines (photographed black) are shown. Mesophyll enclosed by these lines resembled that outside them. ×1. *B*, Like *A*, but photographed with a brown filter so that the brown lines and spots photographed white. Parts of lamina occupied by oil 21 photographed gray, while the normal, nonoily parts of the lamina photographed black. This leaf was photographed 8 days after oiling. In contrast, it showed no oily translucence 732 days after oiling. ×80/107. *C*, Oil-canker line separating living and dead bark in an apple limb 219 days after nearly 10 cc of oil 24 saturated with Oil Red O had been injected into this limb through a glass tube. The canker line formed nearly 180 days after the oil injection, and the leaves on this limb died 275 days after this oil injection. ×2. *D*, Oil (black) between the receptacle-cortex cells of an apple fruit 2 days after oil 24 saturated with Oil Red O had twice filled the calyx basin of this apple in the laboratory. ×149.

days. They were sectioned freehand or with a microtome. Most of the sections were cut from living tissues, but some pieces of leaves were frozen with solid carbon dioxide or in water. Sections were then (1) washed in running water, (2) immersed in a 60-percent solution of pyridine for a few minutes to remove chlorophyll and prepare them for staining, (3) stained for a few hours in Oil Red O dissolved in a 70-percent solution of pyridine, (4) destained for a few minutes in 60-percent pyridine, (5) washed in running water for a few minutes, (6) counterstained with light green SF, (7) mounted on slides in pure glycerine, and (8) sealed on the slides with clear Duco varnish.

Oils 3, 13, and 24 were injected into apple limbs and trunks to study the distribution and effects of large amounts of the oils. Holes 1 to 2 cm deep and 3 or 7 mm in diameter were bored into 90 apple limbs and trunks. Glass tubes 8 to 25 cm long and 3 or 7 (mostly 7) mm in diameter were set in the holes and sealed with grafting wax (pl. 2, *H*). The larger tubes had a capacity of 0.36 cc per 1 cm while the smaller tubes had a capacity of 0.16 cc per 1 cm. Additional quantities of oils were placed in the tubes 6 to 9 times during 2 weeks after starting. Oils containing 0.2 percent Oil Red O were used in most of the tubes to make easy the detection of the oils in the trees, but unstained oils were used in many tubes.

L-shaped iron tubes with inner diameters of 6 mm, outer diameters of 11 mm, and sides 8 to 10 cm long were set in holes 2 to 3 cm deep in 22 apple limbs and trunks. Reservoir holes 3 to 6 mm deep and 3 mm in diameter were bored in the bottom of each hole. The tubes were filled with measured amounts (5 to 9 cc) of oil 24 saturated with Oil Red O, and the free ends were covered with glass vials.

EXPERIMENTAL RESULTS

OIL PENETRATION INTO LEAVES

The oils shown in figure 1 entered living leaves of apple, *Pyrus malus* L., and caused translucence by filling many of the intercellular spaces. Hypophyllous applications of oils to normal leaves at 15 to 25° C. revealed the following rates of penetration causing immediate translucence: 2 to 60 seconds for the oils with viscosities of 50 to 108 seconds, and 5 to 30 minutes for oil 8 with a viscosity of 410 seconds. Thus, oils with a low viscosity penetrated more rapidly than those with a high viscosity.

During penetration, the oils inside the leaves commonly spread beyond the margins of the superficial drops of oils. The translucence of the oily spots varied much apparently because different amounts of oils occupied the intercellular spaces in the different vein-bounded polyhedrons of mesophyll. These oily, vein-bounded spots in the leaves were distributed discontinuously, or occupied large regions, many of which surrounded nonoily parts of mesophyll (pl. 1, *B*). This caused the oily sign, angular mottling (pl. 1, *A*). Angular mottling was caused by undiluted oils, and by calcium-caseinate emulsions of 2 to 16 percent of oils that probably penetrated the leaves as free oils. The amount of oil penetrating determined the size and translucence of the angular mottled spots.

Oily spots in living green leaves and in dried specimens of such leaves were translucent and yellowish green by transmitted light, and were blackish green by reflected light in contrast to the nonoily

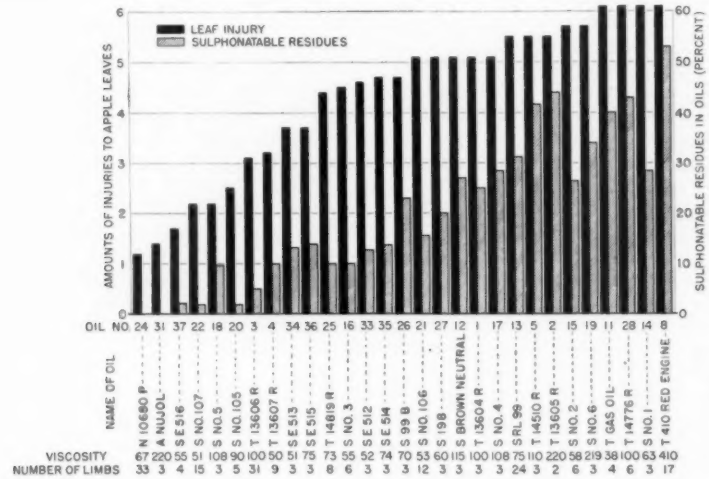


FIGURE 1.—Injuries to apple leaves caused by oils as compared with the sulphonatable residues of the oils. Amounts of oil injuries to leaves shown by solid bars are indicated by numbers that signify as follows: 1, No necrotic symptoms appeared within 22 days after the leaves were oiled, and the bark on the attached twigs was alive from 85 to 145 days after their leaves were oiled; some of the oiled leaves showed no necrotic symptoms within 30 to 60 days. 2, Some mild, necrotic symptoms appeared within 8 to 22 days, and the bark on the twigs was alive from 85 to 145 days after the attached leaves were oiled. 3 like 2, except the leaf symptoms were more abundant and severe. 4, Some severe necrotic symptoms appeared within 8 days, and part of the bark on the attached twigs died within 100 to 145 days. 5, Severe and abundant necrotic symptoms appeared within 8 days, and much of the bark on the attached twigs died within 85 to 145 days. 6, Many oily parts of leaves died within 1 day, or part of the bark on the attached twigs died within 39 days. The shaded bars show the percentage of sulphonatable residue in each oil as determined by the makers of the oils, except oils 5, 13, 15, 16, and 21, the sulphonatable residues of which were determined by Green (*5*) who also used these spray oil numbers. Below are given serial numbers of the petroleum oils, viscosity of each oil is seconds (Saybolt universal viscosimeter) as determined by the oil companies, number of limbs on the leaves of which sufficient oils were placed to make 20 to 90 percent of the laminae translucent, and names or company numbers of the oils, with symbols indicating the names of the oil companies, which signify as follows: N, is L. Sonneborn Sons, Inc., New York; A, is Stanco, Inc.; I, is Standard Oil Co., of California; S, is Shell Oil Co.

parts of the same leaves. Thus, light was absorbed less in refraction and more in reflection by the oily spots than by the nonoily parts.

The appearance of the oily spots in leaves became nearly stable within 2 to 60 minutes after oil penetration started. Thereafter, the sizes, shapes, colors, and translucence of the oily spots in the

EXPLANATORY LEGEND FOR PLATE 2

The oils appear black in these photographs.

A.—Section of an apple limb several centimeters below a glass tube through which nearly 5 cc of oil 24 saturated with Oil Red O was injected 219 days previously. The large, red-oily sector shows where much oil entered the xylem. ×1.

B.—Oil in tracheae of an apple-leaf petiole 32 days after oil 24 was placed on its lamina. ×293.

C.—Oil between the cells of an apple fruit 11 days after oil 8 was painted on the epidermis. ×123.

D.—Raised, yellow blisters surrounding brown depressions around the lenticels in apple bark 213 days after oil 3 saturated with Oil Red O was injected into this limb through a glass tube. The limb then bore normal leaves. ×$\frac{5}{6}$.

E.—Oil inside tracheae and phloem of a 2-year-old apple twig 109 days after the attached leaves were sprayed with 16-percent oil 24 emulsified with calcium caseinate. The oil had passed from the leaves into this twig. ×93.

F.—Oil inside and between parenchyma cells of an apple-leaf petiole 32 days after oil 24 was placed on the attached lamina. ×85.

G.—Oil in the tracheae and in the epiphyllous and hypophyllous parenchyma cells of the midrib of an apple leaf 30 days after oil 8 was applied to its lamina. ×137.

H.—Raised, interlenticel, yellow and gray blisters near the ink label on the bark of this apple trunk into which nearly 180 cc of oil 24 saturated with Oil Red O entered through four glass tubes (one shown at the top) 220 days previously. The oil was injected on October 25 to November 3, 1930. A field of *Cytospora* pyenidia (at top) appeared in bark killed by oil. ×$\frac{1}{4}$.

I.—Yellow blisters in the trunk bark of an apple tree 220 days after oil 24 saturated with Oil Red O was injected into the trunk 34 cm above them. ×$\frac{5}{6}$.

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

living leaves changed very little. This indicated that only negligible amounts of oils evaporated from the interiors of living leaves that had been treated with oils having viscosities of 50 to 410 seconds.

This conclusion that oils disappear only slowly from the interiors of leaves was supported by observations on many oil-mottled leaves that were dried and stored in the herbarium. During the first 1 to 3 years, the oily spots in these dried leaves commonly retained essentially the appearances that they had while the leaves were alive. The following data give the periods of time during which parts of many of the dried leaves remained prominently oily translucent by transmitted light, and black by reflected light: Oil 3, 5.5 years; oils 8, 13, and 21, 4.3 years (pl. 1, *A*, *B*); oil 24, 3.3 years; and oils 1, 5, 12, 26, and 36, 2.4 years. Those of the herbarium specimens of leaves that contained the most viscous oils showed the most numerous, large, and prominently oily spots.

Hypophyllous strips were taken from mature and juvenile apple leaves on several dates and were placed immediately in distilled water. Prompt microscopical examinations showed that the stomatal orifices had dimensions of 10μ to 30μ by 2μ to 7μ, while the surrounding pairs of guard cells had dimensions of 27μ to 38μ by 24μ to 34μ. Thus, the stomatal orifices were large enough to permit rapid flow of oils through them.

OILS IN LEAVES AND TWIGS

Ten oils (table 1) penetrated and spread similarly, so their range of 50 to 108 seconds in viscosities made no apparent difference. These oils occupied the tissues described in table 1 and illustrated in plates 1, *D*, and 2, *B*, *E*, *F*, *G*.

Similar distribution of most of these oils was observed after the leaves were sprayed with emulsions containing 2, 4, 8, and 16 percent of oils. The different concentrations of oil placed on the leaves affected only the amounts of oil in the tissues, as more oil entered the tissues from the undiluted oil than from the emulsions. The globules and distorted masses of oils seen in apple tissues usually were 8μ to 70μ in diameter. No oils were found in any of the unoiled tissues studied.

TABLE 1.—*Locations of petroleum oils inside and between cells of apple leaves and their attached petioles and limbs after the undiluted oils had been painted on the leaves* [1]

General location of oils	Oils in leaf laminae				Oils in attached petioles		Oils that passed from oiled leaves into attached limbs 1 to 4 years old					
	Spongy parenchyma	Palisade parenchyma	Vein tracheae	Vein parenchyma	Tracheae	Parenchyma	Pith	Tracheae	Medullary rays	Phloem	Cortical parenchyma	Lenticel parenchyma
Inside cells	O	O	A	CP	A	CP	CP	A	CP	BC	BC	O
Between cells	A	CP	O	CP	O	CP	A	O	O	CP	A	CP

[1] Relative amounts of oils in and between cells are indicated by letters that signify as follows: O, no oil seen; BC, oils seen in or between some cells; CP, oils commonly present; A, oils abundant. The distribution of the following oils in these tissues were studied: Oils 3, 5, 13, 18, 20, 21, 22, 24, 25, and 28 (fig. 1).

Typical Examples of Oils in Apple Tissues

An ammonia-casein emulsion of 8-percent oil 21 was sprayed on apple leaves on June 6, 1930. Sections of the leaves, cut 34 days later, showed oil inside the vein tracheae and some vein parenchyma cells, and between the phloem, spongy, and palisade parenchyma cells of the veins. Sections of the attached 1930 stem showed oil between the parenchyma cells near the bast, between collenchyma, lenticel, and pith cells; and inside pith cells, tracheae, tracheids, and some phloem cells.

A calcium caseinate emulsion of 16-percent oil 24 was sprayed on apple leaves on seven limbs on July 12, 1929. This oil was found 488 days later in the attached stems inside the tracheae, tracheids, pith, parenchyma, and medullary ray cells; and between pith, phloem, and cortical parenchyma cells. Some oil was found in the 1927 wood. Later sections showed some oil in 1930 wood, showing that the oil was moved from the old wood into the new wood.

An apple leaf was sectioned 7 days after oil 24 was placed on it. The oil was seen in the substomatal cavities, between chlorenchyma cells, and inside vein tracheae and vein parenchyma cells. Sections of the attached twig revealed oil in tracheae, and in and between cortical parenchyma and pith cells.

Apple twigs were sectioned 47 to 77 days after oil 3 was placed on the attached leaves. The oil was seen inside wood tracheae formed during the last 4 years; inside pith, cortical parenchyma, and old phloem cells; and between collenchyma and cortical parenchyma cells.

Oil 8, placed on apple leaves, was later found in the following tissues: Inside the tracheae, and inside and between the parenchyma cells of leaf veins and a midrib (pl. 2, *G*); between the spongy parenchyma cells of leaves; and inside the tracheae and some parenchyma cells of attached twigs. Like the other oils, this very viscous oil also was distributed in the tissues.

Sections showed that the thick-walled cylinders of cells were incomplete on most leaf veins, and were apparently inadequate barriers to the movement of large amounts of oils from the intercellular spaces of leaves into their tracheae. Large amounts of oils commonly passed from leaves into their petioles and twigs.

Distribution and Effect of Large Amounts of Oils in Limbs

The effects of large quantities of oils in tissues were studied by separately injecting oils 3, 13, and 24 through glass tubes into apple limbs and trunks during October 1930. Five to fifty (usually 10 to 25) cubic centimeters of oil passed from each tube into its limb or trunk. The bark usually became oily within 10 to 15 cm from the glass tubes. Oil 13 killed the injected limbs within a few weeks, while oils 3 and 24 killed the injected limbs within 6 to 10 months. Similar results followed the use of stained and unstained oils. Check holes in limbs did not seriously injure the limbs.

On June 5, 1930, oil 24 with the Oil Red O stain was placed directly in drill holes in eight apple limbs, without using glass or iron tubes; 8 to 10 cubic centimeters of oil passed into each limb.

Ten limbs and three trunks were sawed 139 days after oiling in the June series, and 9 to 12 months after oiling in the October series. When oil was not visible immediately on cut surfaces, they were made

smoother and observed 15 minutes later to permit oil present in the tracheae to flow out and become apparent. In these limbs and trunks, red-stained oils were found in the wood 5 to 88 (average of 48) cm below the oil-injection holes (pl. 2, *A*). Oil was found 51 cm above one oil hole. The oils in the limbs occurred mainly in the tracheae, especially in the tracheae and sides of annual rings vertically connected with the oil-injection holes.

Oil 3 was injected through a glass tube into a limb. Sections 10 cm beyond the glass tube were cut 8 days later. They showed oil in tracheae, in and betweeen cortical parenchyma cells, and between collenchyma cells.

Compressed air was used to facilitate oil penetration in other tests. Six of the L-shaped iron tubes were set in holes in apple limbs on June 3 and 20, 1932. Oil 24 saturated with Oil Red O was placed in each tube. Air pressures of 10 to 25 pounds per square inch were applied to each tube, and the compressed air forced 1 to 3 cc of oil into each limb within 7 to 60 minutes. Thus, the compressed air greatly accelerated the penetration of the oil and was the best method of injecting oil into apple limbs. The limbs were sawed 10 to 267 minutes later; red oil was revealed in the tracheae 9 to 49 cm above and below the iron tubes. The oil was conspicuous only in the annual rings vertically connected with the oil reservoirs of the iron tubes. The oil occurred mainly in the inner tracheae of 2 annual rings in 1 limb, and mainly in the central tracheae of 2 annual rings in 2 other limbs, thus showing the tracheae functioning at this time.

OIL PENETRATION INTO APPLE FRUITS

Previous experiments showed that some sprays of oil emulsions caused necrotic spots in apple fruits. Evidently oils entered these apples, so experiments were conducted to test the penetration of apple fruits by oils.

Oils 3, 8, 13, and 24 were placed separately as drops on many apples in the orchard. They soon made oily spots that, by reflected light, were darker colored than adjacent unoiled regions. Oils 3 and 24 caused no necrotic symptoms, while oils 8 and 13 caused blackening and browning of cells in and near the oiled lenticels.

Oils 3 and 24, saturated with Oil Red O, were placed separately as drops on 50 green apples in the orchard. These oils entered through the lenticels and caused oily spots in the apples within 5 to 60 minutes. Strips of oiled epidermis from these apples showed that the lenticels were oily red while the remainder of the epidermis was hyaline. Parenchyma cells and some tracheae within 1 mm of the epidermis were red with oil. Most of the oil was between the parenchyma cells (pl. 1, *D* and 2, *C*).

The stem cavities and calyx basins of 46 ripe Hibernal, Wealthy, and Winesap apples were filled 2 to 5 times with oils 3 and 24 (with and without Oil Red O), used separately in four series of experiments in the laboratory. Each apple absorbed traces to 5 cc of oil within 1 to 4 weeks. Open calyx tubes and orifices around the stems in some apples permitted oils to flow rapidly into the core cavities. No difference was observed in the behavior of the stained and unstained oils. Sections of the oiled apples were examined within 5 to 30 (mostly 7 to 10) days after oiling. Each oiled apple showed a

cone-shaped, very oily layer of tissues 3 to 8 mm thick adjacent to, and below, the oiled stem cavities and calyx basins. Such layers had abundant oil in and between the parenchyma cells (pl. 1, *D*). Some core cavities contained much oil. Some vessels radiating from oily cores were pink with red-stained oils, and oils were seen in some tracheae.

SYMPTOMS CAUSED BY UNDILUTED OILS IN APPLE LEAVES, BARK, AND BUDS

Brown and Dry Green Spots Preceding Defoliation

Oily translucent mesophyll (pl. 1, *B*) commonly died, became dry, and turned dark brown or light green. Dark brown or purple irregular lines or concentric-arc lines 0.5 to 2 mm wide appeared mostly within 39 days and bounded many of these spots (pl. 1, *A*). Some of the purple and brown borders were bounded by nonnecrotic, oily translucent mesophyll. Angular mottling commonly occurred in other parts of the laminae (pl. 1, *B*). The unsulphonatable oil 24 required 3 to 9 weeks to cause these symptoms, while most of the other oils caused such brown spots within 0.5 to 2 weeks. Indophenol blue apparently increased the toxicity of oil 24. Leaves with 50 to 90 percent of the laminae killed by oils usually fell off their trees one to many weeks before normal defoliation. The rapidity of appearance and the abundance of the necrotic leaf spots was directly correlated with the percentages of the sulphonatable residues in the oils (fig. 1).

Epiphyllous Purple Spots

Oil 18 and the oils more than 13 percent sulphonatable (except oils 17, 28, 34, and 36) caused prominent epiphyllous purple spots. Evidently only these oils contained sufficient concentrations of the chemicals that cause this symptom. Thus, this symptom distinguished many oils that were very toxic to apple leaves.

White and Light Brown Epiphyllous Leaf Spots

Oil 11 caused irregular, epiphyllous, white to light brown, usually purple-bordered spots 0.5 to 2 cm wide within 39 days. Some of these spots were hypophyllously green and apparently alive, but most of them were necrotic and hypophyllously dark brown. The fact that only oil 11 caused these symptoms indicates that only it contained the causal chemical in sufficiently concentrated form.

Flavescence

Unlike chlorosis, flavescence describes the abnormal, nonnecrotic, light green to yellow colors in leaves that never were normally green. Flavescent, dwarfed leaves were developed by buds on several limbs girdled by oils 3 and 24 that were injected through glass tubes during the preceding October. Also, slender, rolled, dwarfed, flavescent leaves were developed by the buds of three branches, the leaves of which had been sprayed with 8- and 16-percent oil emulsions 10 and 22 months previously. Most of the flavescent leaves died within a few weeks after appearing.

Leaf Dwarfing and Drying

Leaves delayed in developing, often flavescent, and only 0.5 to 2 cm long, were produced by many buds 60 days after treatment with oils 3 and 24, and by many buds on some limbs injured 6 months previously by these oils injected through glass tubes. Leaves 0.5 to 5 cm long were produced by 60 other limbs into which oils were injected through glass tubes. These leaves were normally green when they appeared, but during the summer they developed similar symptoms of chlorosis, wilting, and drying, and all of them died 2 to 5 months earlier than the leaves on normal limbs. Most of the dried leaves remained attached to their branches during the remainder of the summer. The dying of the leaves usually accompanied the dying of the bark on their branches.

Six of the oils applied in concentrations of 16, 90, and 100 percent killed the leaves on 25 limbs without causing abscission. These leaves became brown and dry, later turned gray, and thereafter remained attached to their branches during 1 to 6 months.

Buds and Twigs Killed by Oil

The buds on 37 dormant limbs were treated separately with oils 3, 12, 15, 16, and 24. Oils 3 and 24 killed a few of the buds on each limb and caused dwarfing of the leaves formed by some of the other buds. In contrast, the more sulphonatable oils 12, 15, and 16 killed most of the treated buds.

The oils (fig. 1) applied in concentrations of 8, 16, 90, and 100 percent to apple leaves entered and killed 150 of the attached twigs within 6 months. For example, oils 3 and 24 killed the tips of such twigs within 3 to 6 months, while the more sulphonatable oil 13 killed large parts of the small limbs within 2 to 7 weeks. These data emphasize the conclusion that the toxicity of an oil is closely correlated with its sulphonatable residue.

Oil Cankers and Narrow Canker Lines in Bark

Narrow canker lines separating living and dead bark developed within 3 to 9 months in nearly half of the 150 oil-killed twigs. Many oil cankers were conspicuous during 1 to 2 months before the canker lines appeared. Two to five canker lines appeared in each of several twigs.

In May 1931 the bark looked normal surrounding the glass tubes through which oils were injected into 90 limbs in October 1930. These limbs developed normal-appearing new leaves. During June to September 1931, cankers which were 5 to 70 cm long and which girdled their limbs developed in the bark around all these glass tubes. Cracks separating living and dead bark formed in 20 of these cankers (pl. 1, *C*) while 4 of the cankers lacked distinct margins. The mature cankers were depressed, with green, gray, orange, or yellow outer surfaces. Within 1 to 8 weeks after girdling cankers developed, the bark shriveled and the leaves withered beyond the cankers.

Yellow and Gray Blisters in Bark of Limbs

Yellow and gray blisters 1 to 3 mm high and 5 to 15 mm wide formed on 17 cankers caused by oils near glass tubes (pl. 2, *D*, *H*, *I*). The groups of blisters were 2 to 15 cm wide and usually were within 5 to

20 cm from the glass tubes. The cortex in the blisters was soft and spongy, with lenticels in the depressions between the blisters. These blisters collapsed within a month, leaving yellow to gray, depressed, cracking cankers from which many parts of the paper-like epidermis separated.

Much oil had entered the bark near the glass tubes. The large amount of oils in the bark tissues presumably decreased the entrance of oxygen and the elimination of water and carbon dioxide. When excess water makes conditions in tissues similarly abnormal, many tissues react by developing intumescences and oedema, as described in the literature. This explanation logically applies to such abnormalities mainly caused by excess oil. Furthermore, by decreasing transpiration, oil might result in excess water in bark tissues and thus cause intumescences.

DISCUSSION

The sizes, shapes, and translucence of oily spots in apple leaves became nearly stable within 2 to 60 minutes after oiling and changed little during the remainder of the growing seasons. Evaporation of the oils from the interiors of living leaves in orchards apparently is negligible. Much of the oil is retained by the leaves and thus is removed from the trees by defoliation. This removal of oils, and the resistance of apple to oils help to explain why there usually is no cumulative injury by oils sprayed on apple leaves.

Low-viscosity oils penetrated apple leaves more rapidly than did high-viscosity oils. The amount of oil penetrating tissues depended on the amount of oil available and the capacity of the tissues. The oils considered here ranged in viscosities from 38 to 410 seconds and were tested in undiluted form. Difference in viscosities was not evidently responsible for different effects of the oils in the apple leaves.

Methods of purifying oils deserve more attention. Oils 21, 33, and 35 were sulphonated with liquid sulphur dioxide in contrast to the comparable oils 34 and 36 that were sulphonated with sulphuric acid. These five oils were stated as having almost identical sulphonatable residues, yet the oils sulphonated with sulphur dioxide were more injurious than those sulphonated with sulphuric acid. Probably sulphuric acid removed more of the toxic chemicals from the oils than did sulphur dioxide. Hence, a statement of the sulphonatable residue of an oil should include a statement of the method of sulphonation. However, the method of sulphonation may be unimportant in commercial sprays with emulsions of only 0.5-percent oil.

Large amounts of oils passed from oil-treated apple leaves into their petioles and stems, as shown by the sections of tissues. The oils were distributed extensively in and between the parenchyma cells, and in the tracheae of leaves, petioles, and twigs. The oils prominently revealed the extensive system of intercellular spaces in the parenchymatous tissues of apple leaves, stems, and fruits. Intercellular spaces are assumed to be physiologically valuable in the distribution of gases in tissues. The diffusion and distribution of water, carbon dioxide, and oxygen is retarded by oils that obstruct stomata and occupy tracheae and intercellular spaces. Hence, oils presumably are physically injurious when they are abundant in apple tissues.

The physical and chemical effects of the oils are contrasted in figure 1 in which the chemical or toxic injuries are represented mainly by the cross-lined bars, while the physical injuries are represented mainly by the parts of the solid bars that project above the tops of the cross-lined bars. The percentages of sulphonatable residues did not indicate accurately the toxic effects of some oils. For example, oil 18 was less toxic than its sulphonatable residue indicated. In contrast, oils 14 and 15 were more toxic than their percentages of sulphonatable residues indicated, probably because some of their chemicals were unusually poisonous. However, the oils that were more than 15 percent sulphonatable, and that caused injuries in amounts of 5 to 6 (fig. 1) were too toxic for commercial use in sprays of oil emulsions on apple leaves.

Physical and chemical injuries are best considered to be fundamentally different. For example, protoplasm dies from physical injury when it is heated or ruptured excessively, or when it lacks sufficient oxygen. In contrast, protoplasm dies from chemical injury when poisonous chemicals react with it.

Toxicity is strictly a chemical property of a substance. The toxicity (total amount of chemical effect) of a substance is modified by (1) the resistance of the affected protoplasm, (2) the oil concentration above the maximum amount tolerable, (3) the temperature of the toxic chemical, and (4) the duration of its toxic action on protoplasm. The volatility of a chemical may affect the duration of its action.

Consequently, the toxicity of an oil depends mainly on its chemically active, mostly sulphonatable parts. These parts differ in their effects on apple. The concentration of oil in a spray should not far exceed the resistance of apple protoplasm to the toxic parts of the oil.

The toxic effects of each undiluted oil depended mainly on the percentage and chemical nature of its sulphonatable residue. Quick killing characterized the primary effect of very toxic oils. Tissues tolerated small amounts of toxic oils and larger amounts of nearly nontoxic oils, but they were badly injured by very toxic oils and by large amounts of less toxic oils. Large amounts of oils passing from leaves into their twigs usually killed the twigs within 9 months.

SUMMARY

Large amounts of oils passed from oiled leaves of apple into their twigs. The oils were widely distributed in and between the parenchyma cells, and in the tracheae of leaves and their twigs. Oil was found in an apple stem 488 days after the oil was sprayed on the leaves then attached to this stem.

Oils prominently revealed the extensive system of intercellular spaces in parenchyma tissues. Large amounts of oils in these spaces are physically injurious because the oils retard the diffusion of oxygen and carbon dioxide. Oils in tracheae retard the distribution of water.

Petroleum oils with viscosities of 50 to 108 seconds hypophyllously penetrated apple leaves and made translucent spots within 2 to 60 seconds. The stomatal orifices in the leaves were 10μ to 30μ by 2μ to 7μ, which permitted the oils to flow rapidly into the leaves.

Oily spots in leaves were translucent and yellowish green by transmitted light, and were blackish green by reflected light, in contrast to the nonoily parts of the same leaves. Evidently, light was absorbed less in refraction and more in reflection by the oily spots than by the nonoily parts.

Oils injected through glass tubes into apple limbs were found 9 to 12 months later in annual rings 5 to 88 cm from the points of injection. The oils were mainly in the tracheae of annual rings vertically connected with the points of injection. Pressure injections of red-stained oils into apple limbs revealed the parts of the annual rings then used in conduction.

Drops of oils on apple fruits penetrated through the lenticels and passed between the fruit-parenchyma cells.

The main symptoms caused by oils in apple leaves were: (1) Brown and dry green spots many of which had brown or purple borders; (2) epiphyllous purple and white spots that indicated very toxic oils; (3) leaf dwarfing; (4) leaf dying and dropping.

The main symptoms caused by oils in apple limbs were: (1) Killing of buds, twigs, and bark; (2) narrow canker lines in bark, and (3) yellow and gray blisters in bark.

Toxicity is a chemical property of an oil. The toxicity of an oil to apple is modified by: (1) The resistance of the protoplasm; (2) the oil concentration above the amount tolerable; (3) the temperature; and (4) the duration of oil action on protoplasm. There was no apparent correlation between the amounts of oil injury to apple leaves and oil viscosities ranging from 38 to 410 seconds.

The toxic effects of the oils depend mainly on the percentages of their sulphonatable residues. However, white leaf spots and epiphyllous purple spots were caused only by certain oils, which showed that the chemical natures as well as the percentages of sulphonatable residues influence the symptoms of oil injuries.

Tests with undiluted oils on apple leaves have an immediately practical value. Toxic effects of oils in apple leaves were determined accurately, easily, and quickly by placing drops of each undiluted oil on the lower sides of normal, mature apple leaves. Unsulphonatable oils were used for comparison. The rapidity, severity, and abundance with which symptoms of injuries appeared showed the toxicity of each oil to apple leaves. The oils that killed large parts of leaves within a week were too injurious for use in oil sprays on apple leaves. Thus, the probable injurious effects of an oil can be tested before it is used commercially in sprays on apple leaves.

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